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(54) Title: MAMMALIAN CYTOKINE-LIKE RECEPTOR			
(57) Abstract			
<p>Novel receptor polypeptides, polynucleotides encoding the polypeptides, and related compositions. The polypeptides of the present invention can be used to down-regulate their natural ligands. The polynucleotides and subsequences thereof can be used as diagnostic probes to determine if chromosome 19 is mutated. The antibodies which bind to the polypeptides can be used to purify the receptors and to inhibit the binding of the ligands onto the receptors.</p>			
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MAMMALIAN CYTOKINE-LIKE RECEPTOR

5

BACKGROUND OF THE INVENTION

Proliferation and differentiation of cells of multicellular organisms are controlled by hormones and polypeptide growth factors. These diffusable molecules allow cells to communicate with each other and act in concert to form cells and organs, and to repair and regenerate damaged tissue. Examples of hormones and growth factors include the steroid hormones (e.g. estrogen, testosterone), parathyroid hormone, follicle stimulating hormone, the interleukins, platelet derived growth factor (PDGF), epidermal growth factor (EGF), granulocyte-macrophage colony stimulating factor (GM-CSF), erythropoietin (EPO) and calcitonin.

20

Hormones and growth factors influence cellular metabolism by binding to receptors. Receptors may be integral membrane proteins that are linked to signaling pathways within the cell, such as second messenger systems. Other classes of receptors are soluble molecules, such as the transcription factors.

25

Of particular interest are receptors for cytokines, molecules that promote the proliferation and/or differentiation of cells. Examples of cytokines include erythropoietin (EPO), which stimulates the development of red blood cells; thrombopoietin (TPO), which stimulates development of cells of the megakaryocyte lineage; and granulocyte-colony stimulating factor (G-CSF), which stimulates development of neutrophils. These cytokines are useful in restoring normal blood cell levels in patients suffering from anemia or receiving chemotherapy

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for cancer. The demonstrated *in vivo* activities of these cytokines illustrates the enormous clinical potential of, and need for, other cytokines, cytokine agonists, and cytokine antagonists.

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SUMMARY OF THE INVENTION

The present invention addresses this need by providing a novel mammalian cytokine-like receptor called
10 mammalian Zcytor5, and related compositions and methods. Within one aspect, the present invention provides an isolated human polynucleotide encoding a ligand-binding human receptor polypeptide. The polypeptide comprises a sequence of amino acids containing (a) the amino acid
15 residues of SEQ ID NO: 17, residues 35 to 422 of SEQ ID NO:2; (b) allelic variants of (a); and (c) sequences that are at least 90%, 95% or 99% identical to (a) or (b). In an alternative embodiment, the polypeptide is comprised of amino acid residues 30 to and including amino acid residue
20 422 of SEQ ID NO:2.

The present invention also provides for a polynucleotide encoding another allelic variant of SEQ ID NO: 2 which is a human polypeptide receptor and is defined
25 by SEQ ID NO: 4 in particular the polypeptide comprised of a sequence of amino acids containing (a) the amino acid residues of SEQ ID NO: 18, residues 34 to 425 of SEQ ID NO:4; (b) allelic variants of (a); and (c) sequences that are at least 90%, 95% or 99% identical to (a) or (b). In
30 an alternative embodiment, the polypeptide is comprised of amino acid residues 29 to and including amino acid residue 425 of SEQ ID NO:4.

Other polynucleotides of the present invention
35 encode the amino acid sequence of SEQ ID NO:21 which is a soluble receptor of SEQ ID NO:17 that does not contain a

C-terminus phosphatidylinositol signal sequence; the amino acid sequence of SEQ ID NO: 20 is a Zcytor5 polypeptide of SEQ ID NO:2 having an alternative N-terminus cleavage site; SEQ ID NO: 22 which has an alternative N-terminus cleavage site of the Zcytor5 polypeptide of SEQ ID NO:4; SEQ ID NO:23 which is an amino acid of SEQ ID NO:18 that does not contain a C-terminus phosphatidylinositol signal sequence and the amino acid sequences defined by SEQ ID NOs: 24-31 which are variants of the Zcytor5 polypeptide of SEQ ID NO:4.

Another embodiment of the present invention is a polynucleotide which encodes rat Zcytor5. In particular, a polynucleotide is claimed which encodes a rat polypeptide containing (a) the amino acid sequence of SEQ ID NO: 19 residues 41 to 425 of SEQ ID NO:6; (b) allelic variants of (a); and (c) sequences that are at least 90%, 95% or 99% identical to (a) or (b).

Within a second aspect of the invention there is provided an expression vector comprising (a) a transcription promoter; (b) a DNA segment encoding a secretory peptide and a ligand-binding Zcytor5 receptor polypeptide, containing an amino acid sequence as described above.

Within a third aspect of the invention there is provided a cultured eukaryotic cell into which has been introduced an expression vector as disclosed above, wherein said cell expresses a mammalian Zcytor5 receptor polypeptide encoded by the DNA segment.

Within a fourth aspect of the invention there is provided an isolated polypeptide. The polypeptide comprises a sequence of amino acids containing (a) the amino acid sequence of SEQ ID NO: 17, residues 35 to 422

of SEQ ID NO:2; (b) allelic variants of (a); and (c) sequences that are at least 90%, 95% or 99% identical to (a) or (b). In an alternative embodiment, the polypeptide is comprised of amino acid residues 30 to and including
5 amino acid residue 422 of SEQ ID NO:2.

The present invention also provides for another allelic variant of SEQ ID NO: 2 which is a human polypeptide receptor and is defined by SEQ ID NO: 4 in
10 particular the polypeptide is comprised of a sequence of amino acids containing (a) the amino acid sequence of SEQ ID NO: 18, residues 34 to 425 of SEQ ID NO:4; (b) allelic variants of (a); and (c) sequences that are at least 90%, 95% or 99% identical to (a) or (b). In an alternative
15 embodiment, the polypeptide is comprised of residues 29 to 425 of SEQ ID NO: 4.

Another embodiment of the present invention is a rat Zcytor5 polypeptide containing (a) the amino acid
20 sequence of SEQ ID NO: 19, residues 41 to 425 of SEQ ID NO:6; (b) allelic variants of (a); and (c) sequences that are at least 80% identical to (a) or (b).

Within a further aspect of the invention there
25 is provided a chimeric polypeptide consisting essentially of a first portion and a second portion joined by a peptide bond. The first portion of the chimeric polypeptide consists essentially of a Zcytor5 receptor polypeptide as described above. The invention also
30 provides expression vectors encoding the chimeric polypeptides and host cells transfected to produce the chimeric polypeptides.

The invention also provides a method for
35 detecting a ligand within a test sample, comprising contacting a test sample with a Zcytor5 polypeptide as

disclosed above, and detecting binding of the polypeptide to ligand in the sample. The polypeptide can be membrane bound within a cultured cell, wherein the detecting step comprises measuring a biological response in the cultured cell. Within another embodiment, the polypeptide is immobilized on a solid support.

Within an additional aspect of the invention there is provided an antibody that specifically binds to a polypeptide as disclosed above and an anti-idiotypic antibody of an antibody which specifically binds to a Zcytor5 antibody, also a method for producing an antibody to Zcytor5.

An additional embodiment of the present invention relates to a peptide or polypeptide which has the amino acid sequence of an epitope-bearing portion of a Zcytor5 polypeptide having an amino acid sequence described above. Peptides or polypeptides having the amino acid sequence of an epitope-bearing portion of a Zcytor5 polypeptide of the present invention include portions of such polypeptides with at least nine, preferably at least 15 and more preferably at least 30 to 50 amino acids, although epitope-bearing polypeptides of any length up to and including the entire amino acid sequence of a polypeptide of the present invention described above are also included in the present invention. Examples of said polypeptides are defined by the amino acid sequences of SEQ ID NOS: 32-37.

30

DETAILED DESCRIPTION OF THE INVENTION

The teachings of all of the references cited in the present specification are incorporated in their entirety herein by reference.

35

The term "affinity tag" is used herein to denote a polypeptide segment that can be attached to a second polypeptide to provide for purification or detection of the second polypeptide or provide sites for attachment of the second polypeptide to a substrate. In principal, any peptide or protein for which an antibody or other specific binding agent is available can be used as an affinity tag. Affinity tags include a poly-histidine tract, protein A [Nilsson et al, *EMBO J.* 4:1075 (1985); Nilsson et al., *Methods Enzymol.* 198:3 (1991)], glutathione S transferase [Smith and Johnson, *Gene* 67:31 (1988)], Glu-Glu affinity tag [Grussenmeyer et al., *Proc. Natl. Acad. Sci. USA* 82:7952-4 (1985)], substance P, FLAGTM peptide (Hopp et al., *Biotechnology* 6:1204-10 (1988), streptavidin binding peptide, or other antigenic epitope or binding domain. See, in general, Ford et al., *Protein Expression and Purification* 2: 95-107 (1991). DNAs encoding affinity tags are available from commercial suppliers (e.g., Pharmacia Biotech, Piscataway, NJ).

The term "allelic variant" denotes any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and may result in phenotypic polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequence. The term allelic variant is also used herein to denote a protein encoded by an allelic variant of a gene.

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phenotypic polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequence. The term allelic variant is also
5 used herein to denote a protein encoded by an allelic variant of a gene.

The terms "amino-terminal" and "carboxyl-terminal" are used herein to denote positions within
10 polypeptides. Where the context allows, these terms are used with reference to a particular sequence or portion of a polypeptide to denote proximity or relative position. For example, a certain sequence positioned carboxyl-terminal to a reference sequence within a polypeptide is
15 located proximal to the carboxyl terminus of the reference sequence, but is not necessarily at the carboxyl terminus of the complete polypeptide.

The term "complement/anti-complement pair"
20 denotes non-identical moieties that form a non-covalently associated, stable pair under appropriate conditions. For instance, biotin and avidin (or streptavidin) are prototypical members of a complement/anti-complement pair. Other exemplary complement/anti-complement pairs include
25 receptor/ligand pairs, antibody/antigen (or hapten or epitope) pairs, sense/antisense polynucleotide pairs, and the like. Where subsequent dissociation of the complement/anti-complement pair is desirable, the complement/anti-complement pair preferably has a binding
30 affinity of $<10^9 \text{ M}^{-1}$.

The term "complements of a polynucleotide molecule" is a polynucleotide molecule having a complementary base sequence and reverse orientation as
35 compared to a reference sequence. For example, the

sequence 5' ATGCACGGG 3' is complementary to 5' CCCGTGCAT 3'.

The term "contig" denotes a polynucleotide that
5 has a contiguous stretch of identical or complementary
sequence to another polynucleotide. Contiguous sequences
are said to "overlap" a given stretch of polynucleotide
sequence either in their entirety or alone a partial
stretch of the polynucleotide. For example,
10 representative contigs to the polynucleotide sequence 5'-
ATGGCTTAGCTT-3' are 5'-TAGCTTgagtct-3' and 3'-gtcgacTACCGA-
5'.

The term "degenerate nucleotide sequence"
15 denotes a sequence of nucleotides that includes one or
more degenerate codons (as compared to a reference
polynucleotide molecule that encodes a polypeptide).
Degenerate codons contain different triplets of
nucleotides, but encode the same amino acid residue (i.e.,
20 GAU and GAC triplets each encode Asp).

The term "expression vector" is used to denote a
25 DNA molecule, linear or circular, that comprises a segment
encoding a polypeptide of interest operably linked to
additional segments that provide for its transcription.
Such additional segments include promoter and terminator
sequences, and may also include one or more origins of
30 replication, one or more selectable markers, an enhancer,
a polyadenylation signal, etc. Expression vectors are
generally derived from plasmid or viral DNA, or may
contain elements of both.

35 The term "isolated", when applied to a
polynucleotide, denotes that the polynucleotide has been

removed from its natural genetic milieu and is thus free of other extraneous or unwanted coding sequences, and is in a form suitable for use within genetically engineered protein production systems. Such isolated molecules are those that are separated from their natural environment and include cDNA and genomic clones. Isolated DNA molecules of the present invention are free of other genes with which they are ordinarily associated, but may include naturally occurring 5' and 3' untranslated regions such as promoters and terminators. The identification of associated regions will be evident to one of ordinary skill in the art, for example, Dynan and Tijan, *Nature* 316:774-78 (1985).

An "isolated" polypeptide or protein is a polypeptide or protein that is found in a condition other than its native environment, such as apart from blood and animal tissue. In a preferred form, the isolated polypeptide is substantially free of other polypeptides, particularly other polypeptides of animal origin. It is preferred to provide the polypeptides in a highly purified form, i.e. greater than 95% pure, more preferably greater than 99% pure. When used in this context, the term "isolated" does not exclude the presence of the same polypeptide in alternative physical forms, such as dimers or alternatively glycosylated or derivatized forms.

"Operably linked", when referring to DNA segments, indicates that the segments are arranged so that they function in concert for their intended purposes, e.g. transcription initiates in the promoter and proceeds through the coding segment to the terminator.

The term "ortholog" (or "species homolog") denotes a polypeptide or protein obtained from one species

that has homology to an analogous polypeptide or protein from a different species.

5 The term "paralog" denotes a polypeptide or protein obtained from a given species that has homology to a distinct polypeptide or protein from that same species.

10 A "polynucleotide" is a single- or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. Polynucleotides include RNA and DNA, and may be isolated from natural sources, synthesized in vitro, or prepared from a combination of natural and synthetic molecules.

15 A "polypeptide" is a polymer of amino acid residues joined by peptide bonds, whether produced naturally or synthetically. Polypeptides of less than about 10 amino acid residues are commonly referred to as "peptides".

20

The term "promoter" is used herein for its art-recognized meaning to denote a portion of a gene containing DNA sequences that provide for the binding of RNA polymerase and initiation of transcription. Promoter sequences are commonly, but not always, found in the 5' non-coding regions of genes.

30 A "protein" is a macromolecule comprising one or more polypeptide chains. A protein may also comprise non-peptidic components, such as carbohydrate groups. Carbohydrates and other non-peptidic substituents may be added to a protein by the cell in which the protein is produced, and will vary with the type of cell. Proteins are defined herein in terms of their amino acid backbone structures; substituents such as carbohydrate groups are
35 generally not specified, but may be present nonetheless.

The term "receptor" denotes a cell-associated protein that binds to a bioactive molecule (i.e., a ligand) and mediates the effect of the ligand on the cell.

5 Membrane-bound receptors are characterized by a multi-domain structure comprising an extracellular ligand-binding domain and an intracellular effector domain that is typically involved in signal transduction. Binding of ligand to receptor results in a conformational change in

10 the receptor that causes an interaction between the effector domain and other molecule(s) in the cell. This interaction in turn leads to an alteration in the metabolism of the cell. Metabolic events that are linked to receptor-ligand interactions include gene

15 transcription, phosphorylation, dephosphorylation, increases in cyclic AMP production, mobilization of cellular calcium, mobilization of membrane lipids, cell adhesion, hydrolysis of inositol lipids and hydrolysis of phospholipids. In general, receptors can be membrane

20 bound, cytosolic or nuclear; monomeric (e.g., thyroid stimulating hormone receptor, beta-adrenergic receptor) or multimeric (e.g., PDGF receptor, growth hormone receptor, IL-3 receptor, GM-CSF receptor, G-CSF receptor, erythropoietin receptor and IL-6 receptor).

25

The term "secretory signal sequence" denotes a DNA sequence that encodes a polypeptide (a "secretory peptide") that, as a component of a larger polypeptide, directs the larger polypeptide through a secretory pathway

30 of a cell in which it is synthesized. The larger polypeptide is commonly cleaved to remove the secretory peptide during transit through the secretory pathway.

The term "splice variant" is used herein to

35 denote alternative forms of RNA transcribed from a gene. Splice variation arises naturally through use of

alternative splicing sites within a transcribed RNA molecule, or less commonly between separately transcribed RNA molecules, and may result in several mRNAs transcribed from the same gene. Splice variants may encode
5 polypeptides having altered amino acid sequence. The term splice variant is also used herein to denote a protein encoded by a splice variant of an mRNA transcribed from a gene.

10 A "soluble receptor" is a receptor polypeptide that is not bound to a cell membrane. Soluble receptors are most commonly ligand-binding receptor polypeptides that lack transmembrane and cytoplasmic domains. Soluble receptors can comprise additional amino acid residues,
15 such as affinity tags that provide for purification of the polypeptide or provide sites for attachment of the polypeptide to a substrate, or immunoglobulin constant region sequences. Many cell-surface receptors have naturally occurring, soluble counterparts that are
20 produced by proteolysis or translated from alternatively spliced mRNAs. Receptor polypeptides are said to be substantially free of transmembrane and intracellular polypeptide segments when they lack sufficient portions of these segments to provide membrane anchoring or signal
25 transduction, respectively.

The present invention is based in part upon the discovery of a novel DNA sequence that encodes a protein having the structure of a cytokine receptor, including the
30 conserved WSXWS motif. Analysis of the tissue distribution of the mRNA corresponding to this novel DNA showed that expression was present in highest amounts in placenta, thyroid, heart and skeletal muscle with lower levels in prostate and trachea.

Cytokine receptors subunits are characterized by a multi-domain structure comprising a ligand-binding domain and an effector domain that is typically involved in signal transduction. Multimeric cytokine receptors include homodimers (e.g., PDGF receptor $\alpha\alpha$ and $\beta\beta$ isoforms, erythropoietin receptor, MPL [thrombopoietin receptor], and G-CSF receptor), heterodimers whose subunits each have ligand-binding and effector domains (e.g., PDGF receptor $\alpha\beta$ isoform), and multimers having component subunits with disparate functions (e.g., IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, and GM-CSF receptors). Some receptor subunits are common to a plurality of receptors. For example, the AIC2B subunit, which cannot bind ligand on its own but includes an intracellular signal transduction domain, is a component of IL-3 and GM-CSF receptors. Many cytokine receptors can be placed into one of four related families on the basis of their structures and functions. Hematopoietic receptors, for example, are characterized by the presence of a domain containing conserved cysteine residues and the WSXWS motif. Additional domains, including protein kinase domains; fibronectin type III domains; and immunoglobulin domains, which are characterized by disulfide-bonded loops, are present in certain hematopoietic receptors. Cytokine receptor structure has been reviewed by Urdal, *Ann. Reports Med. Chem.* 26:221-228 (1991) and Cosman, *Cytokine* 5:95-106 (1993). It is generally believed that under selective pressure for organisms to acquire new biological functions, new receptor family members arose from duplication of existing receptor genes leading to the existence of multi-gene families. Family members thus contain vestiges of the ancestral gene, and these characteristic features can be exploited in the isolation and identification of additional family members. The cytokine receptor superfamily is subdivided as shown in Table 1.

Table 1Cytokine Receptor Superfamily

5	Immunoglobulin family
	CSF-1 receptor
	MGF receptor
	IL-1 receptor
	PDGF receptor
10	Hematopoietin family
	erythropoietin receptor
	G-CSF receptor
	IL-2 receptor b-subunit
	IL-3 receptor
15	IL-4 receptor
	IL-5 receptor
	IL-6 receptor
	IL-7 receptor
	IL-9 receptor
20	GM-CSF receptor a-subunit
	GM-CSF receptor b-subunit
	Prolactin receptor
	CNTF receptor
	Oncostatin M receptor
25	Leukemia inhibitory factor receptor
	Growth hormone receptor
	MPL
	Leptin receptor
	TNF receptor family
30	TNF (p80) receptor
	TNF (p60) receptor
	TNFR-RP
	CD27
	CD30
35	CD40
	4-1BB

Table 1, continued

OX-40

Fas

NGF receptor

5

Other

IL-2 receptor α -subunitIL-15 receptor α -subunitIFN- γ receptor

10

Cell-surface cytokine receptors are further characterized by the presence of additional domains. These receptors are anchored in the cell membrane by a transmembrane domain characterized by a sequence of hydrophobic amino acid residues (typically about 21-25 residues), which is commonly flanked by positively charged residues (Lys or Arg). On the opposite end of the protein from the extracellular domain and separated from it by the transmembrane domain is an intracellular domain.

20

The novel receptor of the present invention was initially identified by the presence of the conserved WSXWS motif. Analysis of a human cDNA clone encoding human Zcytor5 (SEQ ID NO:1) revealed an open reading frame encoding 422 amino acids (SEQ ID NO:2) or an allelic variant reveals an open reading of 425 amino acid residues, SEQ ID NO: 3 and SEQ ID NO:4.

25

Within preferred embodiments of the invention the isolated polynucleotides will hybridize to similar sized regions of SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:5, or a sequence complementary thereto, under stringent conditions. In general, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence

30

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hybridizes to a perfectly matched probe. Typical stringent conditions are those in which the salt concentration is at least about 0.02 M at pH 7 and the temperature is at least about 60°C. As previously noted, the isolated polynucleotides of the present invention include DNA and RNA. Methods for isolating DNA and RNA are well known in the art. It is generally preferred to isolate RNA from testis, including whole testis tissue extracts or testicular cells, such as Sertoli cells, Leydig cells, spermatogonia, or epididymis, although DNA can also be prepared using RNA from other tissues or isolated as genomic DNA. Total RNA can be prepared using guanidine HCl extraction followed by isolation by centrifugation in a CsCl gradient [Chirgwin et al., *Biochemistry* 18:52-94 (1979)]. Poly (A)⁺ RNA is prepared from total RNA using the method of Aviv and Leder, *Proc. Natl. Acad. Sci. USA* 69:1408-1412 (1972). Complementary DNA (cDNA) is prepared from poly(A)⁺ RNA using known methods. Polynucleotides encoding Zcytor5 polypeptides are then identified and isolated by, for example, hybridization or PCR.

Those skilled in the art will recognize that the sequences disclosed in SEQ ID NOS:1, 2, 3,4 represent single alleles of the human and SEQ ID NOs 5 and 6 of the rat Zcytor5 receptors. Allelic variants of these sequences can be cloned by probing cDNA or genomic libraries from different individuals according to standard procedures.

The present invention further provides counterpart receptors and polynucleotides from other species ("species orthologs"). Of particular interest are Zcytor5 receptors from other mammalian species, including murine, porcine, ovine, bovine, canine, feline, equine,

and other primate receptors. Species orthologs of the human and macaque Zcytor5 receptors can be cloned using information and compositions provided by the present invention in combination with conventional cloning techniques. For example, a cDNA can be cloned using mRNA obtained from a tissue or cell type that expresses the receptor. Suitable sources of mRNA can be identified by probing Northern blots with probes designed from the sequences disclosed herein. A library is then prepared from mRNA of a positive tissue or cell line. A receptor-encoding cDNA can then be isolated by a variety of methods, such as by probing with a complete or partial human or macaque cDNA or with one or more sets of degenerate probes based on the disclosed sequences. A cDNA can also be cloned using the polymerase chain reaction, or PCR (Mullis, U.S. Patent No. 4,683,202), using primers designed from the sequences disclosed herein. Within an additional method, the cDNA library can be used to transform or transfect host cells, and expression of the cDNA of interest can be detected with an antibody to the receptor. Similar techniques can also be applied to the isolation of genomic clones.

The polynucleotides of the present invention can be synthesized using DNA synthesizers. Currently the method of choice is the phosphoramidite method. If chemically synthesized double stranded DNA is required for an application such as the synthesis of a gene or a gene fragment, then each complementary strand is made separately. The production of short genes (60 to 80 bp) can be accomplished by synthesizing the complementary strands and then annealing them. For the production of longer genes (>300 bp), however, special strategies must be invoked, because the coupling efficiency of each cycle during chemical DNA synthesis is seldom 100%. To overcome this problem, synthetic genes (double-stranded) are

assembled in modular form from single-stranded fragments that are from 20 to 100 nucleotides in length.

One method for building a synthetic gene
5 requires the initial production of a set of overlapping, complementary oligonucleotides, each of which is between 20 to 60 nucleotides long. The sequences of the strands are planned so that, after annealing, the two end segments of the gene are aligned to give blunt or staggered ends.
10 Each internal section of the gene has complementary 3' and 5' terminal extensions that are designed to base pair precisely with an adjacent section. Thus, after the gene is assembled, the only remaining requirement to complete the process is sealing the nicks along the backbones of
15 the two strands with T4 DNA ligase. In addition to the protein coding sequence, synthetic genes can be designed with terminal sequences that facilitate insertion into a restriction endonuclease sites of a cloning vector and other sequences should also be added that contain signals
20 for the proper initiation and termination of transcription and translation. See Glick, Bernard R. and Jack J. Pasternak, *Molecular Biotechnology, Principles & Applications of Recombinant DNA*, (ASM Press, Washington, D.C. 1994), Itakura, K. et al. Synthesis and use of
25 synthetic oligonucleotides. *Annu. Rev. Biochem.* 53 : 323-356 (1984), and Climie, S. et al. Chemical synthesis of the thymidylate synthase gene. *Proc. Natl. Acad. Sci. USA* 87 :633-637 (1990).

30 Another embodiment of the present invention provides for a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide of the invention. The epitope of the this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide of the
35 invention. A region of a protein to which an antibody can bind is defined as an "antigenic epitope". See for

instance, Geysen, H.M. et al., *Proc. Natl. Acad Sci. USA* 81:3998-4002 (1984).

As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody can bind), it is well known in the art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See Sutcliffe, J.G. et al. *Science* 219:660-666 (1983). Peptides capable of eliciting protein-reactive sera are frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (i.e., immunogenic epitopes) nor to the amino or carboxyl terminals. Peptides that are extremely hydrophobic and those of six or fewer residues generally are ineffective at inducing antibodies that bind to the mimicked protein; longer soluble peptides, especially those containing proline residues, usually are effective.

Antigenic epitope-bearing peptides and polypeptides of the invention are therefore useful to raise antibodies, including monoclonal antibodies, that bind specifically to a polypeptide of the invention. Antigenic epitope-bearing peptides and polypeptides of the present invention contain a sequence of at least nine, preferably between 15 to about 30 amino acids contained within the amino acid sequence of a polypeptide of the invention. However, peptides or polypeptides comprising a larger portion of an amino acid sequence of the invention, containing from 30 to 50 amino acids, or any length up to and including the entire amino acid sequence of a polypeptide of the invention, also are useful for inducing antibodies that react with the protein. Preferably, the

amino acid sequence of the epitope-bearing peptide is selected to provide substantial solubility in aqueous solvents (i.e., the sequence includes relatively hydrophilic residues and hydrophobic residues are preferably avoided); and sequences containing proline residues are particularly preferred. All of the polypeptides shown in the sequence listing contain antigenic epitopes to be used according to the present invention, however, specifically designed antigenic epitopes include the peptides defined by SEQ ID NOs:32-37.

The present invention also provides isolated receptor polypeptides that are substantially identical to the receptor polypeptides of SEQ ID NOs: 2, 4 and 6 and their species orthologs. By "isolated" is meant a protein or polypeptide that is found in a condition other than its native environment, such as apart from blood and animal tissue. In a preferred form, the isolated polypeptide is substantially free of other polypeptides, particularly other polypeptides of animal origin. It is preferred to provide the polypeptides in a highly purified form, i.e. greater than 95% pure, more preferably greater than 99% pure. The term "substantially homologous" is used herein to denote polypeptides having 50%, preferably 60%, more preferably at least 80%, sequence identity to the sequences shown in SEQ ID NO:2, 4, or 7 or their species orthologs. Such polypeptides will more preferably be at least 90% identical, and most preferably 95% or more identical to SEQ ID NO:2, 4 or 6 or their species orthologs. Percent sequence identity is determined by conventional methods. See, for example, Altschul et al., *Bull. Math. Bio.* 48: 603-616 (1986) and Henikoff and Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915-10919 (1992). Briefly, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty

of 10, a gap extension penalty of 1, and the "blossom 62" scoring matrix of Henikoff and Henikoff (ibid.) as shown in Table 2 (amino acids are indicated by the standard one-letter codes). The percent identity is then calculated

5 as:

$$\frac{\text{Total number of identical matches}}{\text{[length of the longer sequence plus the number of gaps introduced into the longer sequence in order to align the two sequences]}} \times 100$$

10

Table 2

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
5	A 4																			
	R -1	5																		
	N -2	0	6																	
	D -2	-2	1	6																
	C 0	-3	-3	-3	9															
	Q -1	1	0	0	-3	5														
10	E -1	0	0	2	-4	2	5													
	G 0	-2	0	-1	-3	-2	-2	6												
	H -2	0	1	-1	-3	0	0	-2	8											
	I -1	-3	-3	-3	-1	-3	-3	-4	-3	4										
	L -1	-2	-3	-4	-1	-2	-3	-4	-3	2	4									
15	K -1	2	0	-1	-3	1	1	-2	-1	-3	-2	5								
	M -1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5							
	F -2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6						
	P -1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7					
20	S 1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4				
	T 0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5			
	W -3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11		
	Y -2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	
	V 0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4

Sequence identity of polynucleotide molecules is determined by similar methods using a ratio as disclosed above.

5 Polynucleotides, generally a cDNA sequence, of the present invention encode the above-described polypeptides. A cDNA sequence which encodes a polypeptide of the present invention is comprised of a series of codons, each amino acid residue of the polypeptide being
10 encoded by a codon and each codon being comprised of three nucleotides. The amino acid residues are encoded by their respective codons as follows.

Alanine (Ala) is encoded by GCA, GCC, GCG or
15 GCT;
Cysteine (Cys) is encoded by TGC or TGT;
Aspartic acid (Asp) is encoded by GAC or GAT;
Glutamic acid (Glu) is encoded by GAA or GAG;
Phenylalanine (Phe) is encoded by TTC or TTT;
20 Glycine (Gly) is encoded by GGA, GGC, GGG or
GGT;
Histidine (His) is encoded by CAC or CAT;
Isoleucine (Ile) is encoded by ATA, ATC or ATT;
Lysine (Lys) is encoded by AAA, or AAG;
25 Leucine (Leu) is encoded by TTA, TTG, CTA, CTC,
CTG or CTT;
Methionine (Met) is encoded by ATG;
Asparagine (Asn) is encoded by AAC or AAT;
Proline (Pro) is encoded by CCA, CCC, CCG or
30 CCT;
Glutamine (Gln) is encoded by CAA or CAG;
Arginine (Arg) is encoded by AGA, AGG, CGA, CGC,
CGG or CGT;
Serine (Ser) is encoded by AGC, AGT, TCA, TCC,
35 TCG or TCT;

Threonine (Thr) is encoded by ACA, ACC, ACG or
ACT;

Valine (Val) is encoded by GTA, GTC, GTG or GTT;

Tryptophan (Trp) is encoded by TGG; and

5 Tyrosine (Tyr) is encoded by TAC or TAT.

It is to be recognized that according to the
present invention, when a cDNA is claimed as described
above, it is understood that what is claimed are both the
10 sense strand, the anti-sense strand, and the DNA as
double-stranded having both the sense and anti-sense
strand annealed together by their respective hydrogen
bonds. Also claimed is the messenger RNA (mRNA) which
encodes the polypeptides of the present invention, and
15 which mRNA is encoded by the above-described cDNA. A
messenger RNA (mRNA) will encode a polypeptide using the
same codons as those defined above, with the exception
that each thymine nucleotide (T) is replaced by a uracil
nucleotide (U).

20

Substantially identical proteins and
polypeptides are characterized as having one or more amino
acid substitutions, deletions or additions. These changes
25 are preferably of a minor nature, that is conservative
amino acid substitutions (see Table 3) and other
substitutions that do not significantly affect the folding
or activity of the protein or polypeptide; small
deletions, typically of one to about 30 amino acids; and
30 small amino- or carboxyl-terminal extensions, such as an
amino-terminal methionine residue, a small linker peptide
of up to about 20-25 residues, or a small extension that
facilitates purification (an affinity tag), such as a
poly-histidine tract, protein A, Nilsson et al., *EMBO J.*
35 4:1075 (1985); Nilsson et al., *Methods Enzymol.* 198:3
(1991), glutathione S transferase, Smith and Johnson,

Gene 67:31 (1988), or other antigenic epitope or binding domain. See, in general Ford et al., *Protein Expression and Purification* 2: 95-107 (1991). DNAs encoding affinity tags are available from commercial suppliers (e.g.,
 5 Pharmacia Biotech, Piscataway, NJ).

Table 3

<u>Conservative amino acid substitutions</u>	
10	Basic: arginine
	lysine
	histidine
15	Acidic: glutamic acid
	aspartic acid
	Polar: glutamine
20	asparagine
	Hydrophobic: leucine
	isoleucine
25	valine
	Aromatic: phenylalanine
	tryptophan
30	tyrosine
	Small: glycine
	alanine
35	serine
	threonine
	methionine

Essential amino acids in the receptor polypeptides of the present invention can be identified
 30 according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis, Cunningham and Wells, *Science* 244: 1081-1085 (1989); Bass et al., *Proc. Natl. Acad. Sci. USA* 88:4498-4502 (1991). In the latter technique, single alanine mutations are
 35 introduced at every residue in the molecule, and the resultant mutant molecules are tested for biological

activity (e.g., ligand binding and signal transduction) to identify amino acid residues that are critical to the activity of the molecule. Sites of ligand-receptor interaction can also be determined by analysis of crystal structure as determined by such techniques as nuclear magnetic resonance, crystallography or photoaffinity labeling. See, for example, de Vos et al., *Science* 255:306-312 (1992); Smith et al., *J. Mol. Biol.* 224:899-904 (1992); Wlodaver et al., *FEBS Lett.* 309:59-64 (1992). The identities of essential amino acids can also be inferred from analysis of homologies with related receptors.

Multiple amino acid substitutions can be made and tested using known methods of mutagenesis and screening, such as those disclosed by Reidhaar-Olson and Sauer, *Science* 241:53-57 (1988) or Bowie and Sauer, *Proc. Natl. Acad. Sci. USA* 86:2152-2156 (1989). Briefly, these authors disclose methods for simultaneously randomizing two or more positions in a polypeptide, selecting for functional polypeptide, and then sequencing the mutagenized polypeptides to determine the spectrum of allowable substitutions at each position. Other methods that can be used include phage display, e.g., Lowman et al., *Biochem.* 30:10832-10837 (1991); Ladner et al., U.S. Patent No. 5,223,409; Huse, WIPO Publication WO 92/06204) and region-directed mutagenesis, Derbyshire et al., *Gene* 46:145 (1986); Ner et al., *DNA* 7:127, (1988).

Mutagenesis methods as disclosed above can be combined with high-throughput screening methods to detect activity of cloned, mutagenized receptors in host cells. Preferred assays in this regard include cell proliferation assays and biosensor-based ligand-binding assays, which are described below. Mutagenized DNA molecules that encode active receptors or portions thereof (e.g., ligand-binding

fragments) can be recovered from the host cells and rapidly sequenced using modern equipment. These methods allow the rapid determination of the importance of individual amino acid residues in a polypeptide of interest, and can be applied to polypeptides of unknown structure.

Using the methods discussed above, one of ordinary skill in the art can prepare a variety of polypeptides that are substantially homologous to residues SEQ ID NOs:2, 4, 6, 17, 18, or 19 or allelic variants thereof and retain the ligand-binding properties of the wild-type receptor.

The receptor polypeptides of the present invention, including full-length receptors, receptor fragments (e.g. ligand-binding fragments), and fusion polypeptides can be produced in genetically engineered host cells according to conventional techniques. Suitable host cells are those cell types that can be transformed or transfected with exogenous DNA and grown in culture, and include bacteria, fungal cells, and cultured higher eukaryotic cells. Eukaryotic cells, particularly cultured cells of multicellular organisms, are preferred. Techniques for manipulating cloned DNA molecules and introducing exogenous DNA into a variety of host cells are disclosed by Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989, and Ausubel et al., *ibid.*

In general, a DNA sequence encoding a Zcytor5 receptor polypeptide is operably linked to other genetic elements required for its expression, generally including a transcription promoter and terminator, within an expression vector. The vector will also commonly contain

one or more selectable markers and one or more origins of replication, although those skilled in the art will recognize that within certain systems selectable markers may be provided on separate vectors, and replication of the exogenous DNA may be provided by integration into the host cell genome. Selection of promoters, terminators, selectable markers, vectors and other elements is a matter of routine design within the level of ordinary skill in the art. Many such elements are described in the literature and are available through commercial suppliers.

To direct a Zcytor5 receptor polypeptide into the secretory pathway of a host cell, a secretory signal sequence (also known as a leader sequence, prepro sequence or pre sequence) is provided in the expression vector. The secretory signal sequence may be that of the receptor, or may be derived from another secreted protein (e.g., t-PA) or synthesized *de novo*. The secretory signal sequence is joined to the Zcytor5 DNA sequence in the correct reading frame. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the polypeptide of interest, although certain signal sequences may be positioned elsewhere in the DNA sequence of interest (see, e.g., Welch et al., U.S. Patent No. 5,037,743; Holland et al., U.S. Patent No. 5,143,830).

Cultured mammalian cells are preferred hosts within the present invention. Methods for introducing exogenous DNA into mammalian host cells include calcium phosphate-mediated transfection, Wigler et al., *Cell* 14:725 (1978); Corsaro and Pearson, *Somatic Cell Genetics* 7:603 (1981), Graham and Van der Eb, *Virology* 52:456 (1973), electroporation, Neumann et al., *EMBO J.* 1:841-845 (1982), DEAE-dextran mediated transfection, Ausubel et al., eds., *Current Protocols in Molecular Biology* (John Wiley and Sons, Inc., NY, 1987), and liposome-mediated

transfection, Hawley-Nelson et al., *Focus* 15:73 (1993);
Ciccarone et al., *Focus* 15:80 (1993). The production of
recombinant polypeptides in cultured mammalian cells is
disclosed, for example, by Levinson et al., U.S. Patent
5 No. 4,713,339; Hagen et al., U.S. Patent No. 4,784,950;
Palmiter et al., U.S. Patent No. 4,579,821; and Ringold,
U.S. Patent No. 4,656,134. Suitable cultured mammalian
cells include the COS-1 (ATCC No. CRL 1650), COS-7 (ATCC
No. CRL 1651), BHK (ATCC No. CRL 1632), BHK 570 (ATCC No.
10 CRL 10314), 293 (ATCC No. CRL 1573; Graham et al., *J. Gen.*
Viro. 36:59-72, (1977) and Chinese hamster ovary (e.g.
CHO-K1; ATCC No. CCL 61) cell lines. Additional suitable
cell lines are known in the art and available from public
depositories such as the American Type Culture Collection,
15 Rockville, Maryland. In general, strong transcription
promoters are preferred, such as promoters from SV-40 or
cytomegalovirus. See, e.g., U.S. Patent No. 4,956,288.
Other suitable promoters include those from
metallothionein genes (U.S. Patent Nos. 4,579,821 and
20 4,601,978,) and the adenovirus major late promoter.

Drug selection is generally used to select for
cultured mammalian cells into which foreign DNA has been
inserted. Such cells are commonly referred to as
25 "transfectants". Cells that have been cultured in the
presence of the selective agent and are able to pass the
gene of interest to their progeny are referred to as
"stable transfectants." A preferred selectable marker is
a gene encoding resistance to the antibiotic neomycin.
30 Selection is carried out in the presence of a neomycin-
type drug, such as G-418 or the like. Selection systems
may also be used to increase the expression level of the
gene of interest, a process referred to as
"amplification." Amplification is carried out by
35 culturing transfectants in the presence of a low level of
the selective agent and then increasing the amount of

- selective agent to select for cells that produce high levels of the products of the introduced genes. A preferred amplifiable selectable marker is dihydrofolate reductase, which confers resistance to methotrexate.
- 5 Other drug resistance genes (e.g. hygromycin resistance, multi-drug resistance, puromycin acetyltransferase) can also be used.

- Other higher eukaryotic cells can also be used
- 10 as hosts, including insect cells, plant cells and avian cells. Transformation of insect cells and production of foreign polypeptides therein is disclosed by Guarino et al., U.S. Patent No. 5,162,222; Bang et al., U.S. Patent No. 4,775,624; and WIPO publication WO 94/06463, which are
- 15 incorporated herein by reference. The use of *Agrobacterium rhizogenes* as a vector for expressing genes in plant cells has been reviewed by Sinkar et al., *J. Biosci. (Bangalore)* 11:47-58 (1987).

- 20 Fungal cells, including yeast cells, and particularly cells of the genus *Saccharomyces*, can also be used within the present invention, such as for producing receptor fragments or polypeptide fusions. Methods for transforming yeast cells with exogenous DNA and producing
- 25 recombinant polypeptides therefrom are disclosed by, for example, Kawasaki, U.S. Patent No. 4,599,311; Kawasaki et al., U.S. Patent No. 4,931,373; Brake, U.S. Patent No. 4,870,008; Welch et al., U.S. Patent No. 5,037,743; and Murray et al., U.S. Patent No. 4,845. Transformed cells
- 30 are selected by phenotype determined by the selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (e.g., leucine). A preferred vector system for use in yeast is the *POT1* vector system disclosed by Kawasaki et al. (U.S. Patent
- 35 No. 4,931,373), which allows transformed cells to be selected by growth in glucose-containing media. Suitable

promoters and terminators for use in yeast include those from glycolytic enzyme genes (see, e.g., Kawasaki, U.S. Patent No. 4,599,311; Kingsman et al., U.S. Patent No. 4,615,974; and Bitter, U.S. Patent No. 4,977,092) and
5 alcohol dehydrogenase genes. See also U.S. Patents Nos. 4,990,446; 5,063,154; 5,139,936 and 4,661,454. Transformation systems for other yeasts, including *Hansenula polymorpha*, *Schizosaccharomyces pombe*, *Kluyveromyces lactis*, *Kluyveromyces fragilis*, *Ustilago*
10 *maydis*, *Pichia pastoris*, *Pichia methanolica*, *Pichia guilliermondii* and *Candida maltosa* are known in the art. See, for example, Gleeson et al., *J. Gen. Microbiol.* 132:3459-3465 (1986) and Cregg, U.S. Patent No. 4,882,279. *Aspergillus* cells may be utilized according to the methods
15 of McKnight et al., U.S. Patent No. 4,935,349. Methods for transforming *Acremonium chrysogenum* are disclosed by Sumino et al., U.S. Patent No. 5,162,228. Methods for transforming *Neurospora* are disclosed by Lambowitz, U.S. Patent No. 4,486,533.

20

Transformed or transfected host cells are cultured according to conventional procedures in a culture medium containing nutrients and other components required for the growth of the chosen host cells. A variety of
25 suitable media, including defined media and complex media, are known in the art and generally include a carbon source, a nitrogen source, essential amino acids, vitamins and minerals. Media may also contain such components as growth factors or serum, as required. The growth medium
30 will generally select for cells containing the exogenously added DNA by, for example, drug selection or deficiency in an essential nutrient which is complemented by the selectable marker carried on the expression vector or co-transfected into the host cell.

35

Within one aspect of the present invention, a novel receptor is produced by a cultured cell, and the cell is used to screen for ligands for the receptor, including the natural ligand, as well as agonists and antagonists of the natural ligand. To summarize this approach, a cDNA or gene encoding the receptor is combined with other genetic elements required for its expression (e.g., a transcription promoter), and the resulting expression vector is inserted into a host cell. Cells that express the DNA and produce functional receptor are selected and used within a variety of screening systems.

Mammalian cells suitable for use in expressing Zcytor5 receptors and transducing a receptor-mediated signal include cells that express a β -subunit, such as the human β_c subunit. In this regard it is generally preferred to employ a cell that is responsive to other cytokines that bind to receptors in the same subfamily, such as IL-3 or GM-CSF, because such cells will contain the requisite signal transduction pathway(s). It is also preferred to use a cell from the same species as the receptor to be expressed. Within a preferred embodiment, the cell is dependent upon an exogenously supplied hematopoietic growth factor for its proliferation. Preferred cell lines of this type are the human TF-1 cell line (ATCC number CRL-2003) and the AML-193 cell line (ATCC number CRL-9589), which are GM-CSF-dependent human leukemic cell lines. In the alternative, suitable host cells can be engineered to produce a β -subunit (e.g., b_c) or other cellular component needed for the desired cellular response. For example, the murine cell line BaF3, Palacios and Steinmetz, *Cell* 41: 727-734 (1985); Mathey-Prevot et al., *Mol. Cell. Biol.* 6: 4133-4135 (1986) or a baby hamster kidney (BHK) cell line can be transfected to express the human b_c subunit (also known as KH97) as well as a Zcytor5 receptor. The latter approach

is advantageous because cell lines can be engineered to express receptor subunits from any species, thereby overcoming potential limitations arising from species specificity. In the alternative, species orthologs of the human receptor cDNA can be cloned and used within cell lines from the same species, such as a mouse cDNA in the BaF3 cell line. Cell lines that are dependent upon one hematopoietic growth factor, such as GM-CSF, can thus be engineered to become dependent upon a Zcytor5 ligand.

10

Cells expressing functional receptor are used within screening assays. A variety of suitable assays are known in the art. These assays are based on the detection of a biological response in a target cell. One such assay is a cell proliferation assay. Cells are cultured in the presence or absence of a test compound, and cell proliferation is detected by, for example, measuring incorporation of tritiated thymidine or by colorimetric assay based on the metabolic breakdown of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Mosman, *J. Immunol. Meth.* 65: 55-63 (1983)). An alternative assay format uses cells that are further engineered to express a reporter gene. The reporter gene is linked to a promoter element that is responsive to the receptor-linked pathway, and the assay detects activation of transcription of the reporter gene. A preferred promoter element in this regard is a serum response element, or SRE, e.g., Shaw et al., *Cell* 56:563-572 (1989). A preferred reporter gene is a luciferase gene, de Wet et al., *Mol. Cell. Biol.* 7:725 (1987). Expression of the luciferase gene is detected by luminescence using methods known in the art (e.g., Baumgartner et al., *J. Biol. Chem.* 269:29094-29101 (1994); Schenborn and Goiffin, *Promega Notes* 41:11 (1993). Luciferase activity assay kits are commercially available from, for example, Promega Corp., Madison, WI. Target cell lines of this type can be

35

used to screen libraries of chemicals, cell-conditioned culture media, fungal broths, soil samples, water samples, and the like. For example, a bank of cell-conditioned media samples can be assayed on a target cell to identify
5 cells that produce ligand. Positive cells are then used to produce a cDNA library in a mammalian expression vector, which is divided into pools, transfected into host cells, and expressed. Media samples from the transfected cells are then assayed, with subsequent division of pools,
10 re-transfection, subculturing, and re-assay of positive cells to isolate a cloned cDNA encoding the ligand.

A natural ligand for the Zcytor5 receptor can also be identified by mutagenizing a cell line expressing
15 the receptor and culturing it under conditions that select for autocrine growth. See WIPO publication WO 95/21930. Within a typical procedure, BaF3 cells expressing Zcytor5 and human b_c are mutagenized, such as with 2-ethylmethanesulfonate (EMS). The cells are then allowed
20 to recover in the presence of IL-3, then transferred to a culture medium lacking IL-3 and IL-4. Surviving cells are screened for the production of a Zcytor5 ligand, such as by adding soluble receptor to the culture medium or by assaying conditioned media on wild-type BaF3 cells and
25 BaF3 cells expressing the receptor.

An additional screening approach provided by the present invention includes the use of hybrid receptor polypeptides. These hybrid polypeptides fall into two
30 general classes. It is preferred that the second receptor be a hematopoietic cytokine receptor, such as mpl receptor (Souyri et al., *Cell* 63: 1137-1147 (1990)). The hybrid receptor will further comprise a transmembrane domain, which may be derived from either receptor. A DNA
35 construct encoding the hybrid receptor is then inserted into a host cell. Cells expressing the hybrid receptor

are cultured in the presence of a ligand for the binding domain and assayed for a response. This system provides a means for analyzing signal transduction mediated by Zcytor5 while using readily available ligands. This
5 system can also be used to determine if particular cell lines are capable of responding to signals transduced by Zcytor5.

Cells found to express the ligand are then used
10 to prepare a cDNA library from which the ligand-encoding cDNA can be isolated as disclosed above. The present invention thus provides, in addition to novel receptor polypeptides, methods for cloning polypeptide ligands for the receptors.

15 Compounds identified as receptor agonists are useful for stimulating proliferation and development of target cells *in vitro* and *in vivo*. For example, agonist compounds are useful as components of defined cell culture
20 media, and may be used alone or in combination with other cytokines and hormones to replace serum that is commonly used in cell culture. Agonists are thus useful in specifically promoting the growth and/or development of testis-derived cells in culture. Agonists and antagonists
25 may also prove useful in the study of spermatogenesis and infertility. Antagonists are useful as research reagents for characterizing sites of ligand-receptor interaction. *In vivo*, receptor agonists may find application in the treatment of male infertility. Antagonists of receptor
30 function may be useful as male contraceptive agents.

The proposed cytokine binding domain of Zcytor5 appears to be closest to the Interleukin-6 β -chain or gp130 (29% identity). The ligand for Zcytor5 is probably
35 a member of the Interleukin-6 family of cytokines which at present includes: Interleukins-6, -11, Leukemia Inhibitory

Factor, Oncostatin M, Cardiotropin-1 and Ciliary
Neurtrophic Factor.

All Zcytor5 cDNAs isolated thus far do not
5 encode a transmembrane domain nor any recognizable
cytoplasmic signaling motifs characteristic of the Class I
receptors. Structurally, Zcytor5 bears close similarity to
 α -subunit of the Ciliary Neurtrophic Factor receptor (CNTF-
R α). It is quite possible Zcytor5 does not have a
10 transmembrane domain form and that the native molecule is
phosphatidyl-inositol linked to the cell membrane in a
manner similar to CNTF-R α .

Rebledo et al. (J. Biol. Chem., 272: 4855-4863)
15 provide evidence for the existence of a third component of
the Cardiotropin-1 receptor (CT-1R). CT-1R is believed to
have a tripartite structure comprised of gp130, gp190 (LIV
Receptor β) and an uncharacterized 45kDa (CT-1R α) subunit
that appears to be linked to the cell surface through a
20 phosphatidyl-inositol linkage. CT-1R α appears to be
important for increased sensitivity and specificity of the
receptor complex to Cardiotropin-1. The data suggests that
Zcytor5 is CT-1R α . Cardiotropin-1 is a member of the
Interleukin-6 family in which gp130 and gp190 are members
25 of a tripartite complex is the Ciliary Neurotrophic Factor
receptor. In this receptor complex, CNTF-R α comprises the
third receptor subunit and it mediates specificity and
high affinity binding of the ligand complex. These
functions are similar to the proposed ones for CT-1R α . One
30 might then argue on the basis of "symmetry of nature" that
CT-1R α would physically resemble CNTF-R α and that the
close structural similarity of Zcytor5 to CNTF-R α would
make Zcytor5 a possible candidate for the third subunit of
CT-1R. Furthermore, the proposed 45 kDa molecular mass of
35 CT-R α agrees with that of Zcytor5 and the transcripts of
CT-1 and Zcytor5 are found in similar tissues. In

particular, both transcripts are found in high levels in heart and in skeletal muscles, which is consistent with the observation that ligand and their receptor subunits are often co-expressed in the same tissue.

5

Cardiotropin-1 was originally cloned by function as a factor involved in cardiac hypertrophy, an adaptive response of heart muscle to an increased work load. Hypertrophy is characterized by reactivation of genes expressed during fetal heart development and by the accumulation of sarcomeric proteins. If Zcytor5 proves to be the subunit that is important in the binding and specificity of Cardiotropin-1 to its receptor, Zcytor5 may prove to be a useful therapeutic antagonist to counteract the hypertrophic response to injury. Cardiotropin-1 has also been shown to promote survival of rat dopaminergic neurons *in vitro*. An agonist-active soluble receptor may potentially be useful in the treatment of neuronal disorders such as Parkinson's disease.

20

Zcytor5 may also be used within diagnostic systems for the detection of circulating levels of ligand. Within a related embodiment, antibodies or other agents that specifically bind to Zcytor5 can be used to detect circulating receptor polypeptides. Elevated or depressed levels of ligand or receptor polypeptides may be indicative of pathological conditions, including cancer.

Zcytor5 receptor polypeptides can be prepared by expressing a DNA encoding a Zcytor5 polypeptide as described in SEQ ID NO:1, 3 and 5. To direct the export of the receptor domain from the host cell, the receptor DNA is linked to a second DNA segment encoding a secretory peptide. It is believed that amino acid 1-34 or in the alternative amino acid residues 1-30 are secretory peptides of SEQ ID NO:2. For SEQ ID NO: 4, it is believed

35

that residues 1-33 or in the alternative 1-29 are secretory peptides. For the rat sequence, it is believed that amino acid residues 1-40 define a secretory peptide. These peptides are generally cleaved after secretion by a mammalian cell. In the alternative, other secretory peptides could be fused to the Zcytor5 polypeptide, such as the t-PA secretory peptide. To facilitate purification of the secreted receptor domain, a C-terminal extension, such as a poly-histidine tag, substance P, Flag™ peptide (Hopp et al., *Biotechnology* 6:1204-1210 (1988); available from Eastman Kodak Co., New Haven, CT) or another polypeptide or protein for which an antibody or other specific binding agent is available, can be fused to the receptor polypeptide.

In an alternative approach, a receptor extracellular domain can be expressed as a fusion with immunoglobulin heavy chain constant regions, typically an F_C fragment, which contains two constant region domains and a hinge region but lacks the variable region. Such fusions are typically secreted as multimeric molecules wherein the Fc portions are disulfide bonded to each other and two receptor polypeptides are arrayed in closed proximity to each other. Fusions of this type can be used to affinity purify the cognate ligand from solution, as an *in vitro* assay tool, to block signals *in vitro* by specifically titrating out ligand, and as antagonists *in vivo* by administering them parenterally to bind circulating ligand and clear it from the circulation. To purify ligand, a Zcytor5-Ig chimera is added to a sample containing the ligand (e.g., cell-conditioned culture media or tissue extracts) under conditions that facilitate receptor-ligand binding (typically near-physiological temperature, pH, and ionic strength). The chimera-ligand complex is then separated by the mixture using protein A, which is immobilized on a solid support (e.g., insoluble

resin beads). The ligand is then eluted using conventional chemical techniques, such as with a salt or pH gradient. In the alternative, the chimera itself can be bound to a solid support, with binding and elution
5 carried out as above. The chimeras may be used *in vivo* to induce infertility. Chimeras with high binding affinity are administered parenterally (e.g., by intramuscular, subcutaneous or intravenous injection). Circulating molecules bind ligand and are cleared from circulation by
10 normal physiological processes. For use in assays, the chimeras are bound to a support via the F_C region and used in an ELISA format.

A preferred assay system employing a ligand-
15 binding receptor fragment uses a commercially available biosensor instrument (BIAcore™, Pharmacia Biosensor, Piscataway, NJ), wherein the receptor fragment is immobilized onto the surface of a receptor chip. Use of this instrument is disclosed by Karlsson, *J. Immunol. Methods* 145:229-240 (1991) and Cunningham and Wells, *J. Mol. Biol.* 234:554-563 (1993). A receptor fragment is covalently attached, using amine or sulfhydryl chemistry, to dextran fibers that are attached to gold film within the flow cell. A test sample is passed through the cell.
20 If ligand is present in the sample, it will bind to the immobilized receptor polypeptide, causing a change in the refractive index of the medium, which is detected as a change in surface plasmon resonance of the gold film. This system allows the determination of on- and off-rates,
25 from which binding affinity can be calculated, and assessment of stoichiometry of binding.
30

Ligand-binding receptor polypeptides can also be used within other assay systems known in the art. Such
35 systems include Scatchard analysis for determination of binding affinity, Scatchard, *Ann. NY Acad. Sci.* 51: 660-

672 (1949) and calorimetric assays, Cunningham et al., *Science* 253:545-548 (1991); Cunningham et al., *Science* 254:821-825 (1991).

5 A receptor ligand-binding polypeptide can also be used for purification of ligand. The receptor polypeptide is immobilized on a solid support, such as beads of agarose, cross-linked agarose, glass, cellulosic resins, silica-based resins, polystyrene, cross-linked
10 polyacrylamide, or like materials that are stable under the conditions of use. Methods for linking polypeptides to solid supports are known in the art, and include amine chemistry, cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation,
15 sulfhydryl activation, and hydrazide activation. The resulting media will generally be configured in the form of a column, and fluids containing ligand are passed through the column one or more times to allow ligand to bind to the receptor polypeptide. The ligand is then
20 eluted using changes in salt concentration or pH to disrupt ligand-receptor binding.

 Zcytor5 polypeptides can also be used to prepare antibodies that specifically bind to Zcytor5 polypeptides.
25 As used herein, the term "antibodies" includes polyclonal antibodies, monoclonal antibodies, antigen-binding fragments thereof such as F(ab')₂ and Fab fragments, and the like, including genetically engineered antibodies. Antibodies are defined to be specifically binding if they
30 bind to a Zcytor5 polypeptide with a K_a of greater than or equal to $10^7/M$. The affinity of a monoclonal antibody can be readily determined by one of ordinary skill in the art (see, for example, Scatchard, *ibid.*).

35 Zcytor5 polypeptides can also be used to prepare antibodies that specifically bind to Zcytor5 polypeptides.

These antibodies can then be used to manufacture anti-idiotypic antibodies. As used herein, the term "antibodies" includes polyclonal antibodies, monoclonal antibodies, antigen-binding fragments thereof such as F(ab')₂ and Fab fragments, and the like, including genetically engineered antibodies. Antibodies are defined to be specifically binding if they bind to a Zcytor5 polypeptide with a K_a of greater than or equal to 10⁷/M. The affinity of a monoclonal antibody can be readily determined by one of ordinary skill in the art (see, for example, Scatchard, *ibid.*).

Methods for preparing polyclonal and monoclonal antibodies are well known in the art (see for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, (Second Edition) (Cold Spring Harbor, NY, 1989); and Hurrell, J. G. R., Ed., *Monoclonal Hybridoma Antibodies: Techniques and Applications* (CRC Press, Inc., Boca Raton, FL, 1982). As would be evident to one of ordinary skill in the art, polyclonal antibodies can be generated from a variety of warm-blooded animals such as horses, cows, goats, sheep, dogs, chickens, rabbits, mice, and rats. The immunogenicity of a Zcytor5 polypeptide may be increased through the use of an adjuvant such as Freund's complete or incomplete adjuvant. A variety of assays known to those skilled in the art can be utilized to detect antibodies which specifically bind to Zcytor5 polypeptides. Exemplary assays are described in detail in *Antibodies: A Laboratory Manual*, Harlow and Lane (Eds.), (Cold Spring Harbor Laboratory Press, 1988). Representative examples of such assays include: concurrent immunoelectrophoresis, radio-immunoassays, radio-immunoprecipitations, enzyme-linked immunosorbent assays (ELISA), dot blot assays, inhibition or competition assays, and sandwich assays.

As would be evident to one of ordinary skill in the art, polyclonal antibodies can be generated by inoculating a variety of warm-blooded animals such as horses, cows, goats, sheep, dogs, chickens, rabbits, mice, hamsters, guinea pigs and rats with a Zcytor5 polypeptide or a fragment thereof. The immunogenicity of a Zcytor5 polypeptide may be increased through the use of an adjuvant, such as alum (aluminum hydroxide) or Freund's complete or incomplete adjuvant. Polypeptides useful for immunization also include fusion polypeptides, such as fusions of Zcytor5 or a portion thereof with an immunoglobulin polypeptide or with maltose binding protein. The polypeptide immunogen may be a full-length molecule or a portion thereof. If the polypeptide portion is "haptene-like", such portion may be advantageously joined or linked to a macromolecular carrier (such as keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) or tetanus toxoid) for immunization.

As used herein, the term "antibodies" includes polyclonal antibodies, affinity-purified polyclonal antibodies, monoclonal antibodies, and antigen-binding fragments, such as $F(ab')_2$ and Fab proteolytic fragments. Genetically engineered intact antibodies or fragments, such as chimeric antibodies, Fv fragments, single chain antibodies and the like, as well as synthetic antigen-binding peptides and polypeptides, are also included. Non-human antibodies may be humanized by grafting non-human CDRs onto human framework and constant regions, or by incorporating the entire non-human variable domains (optionally "cloaking" them with a human-like surface by replacement of exposed residues, wherein the result is a "veneered" antibody). In some instances, humanized antibodies may retain non-human residues within the human variable region framework domains to enhance proper

binding characteristics. Through humanizing antibodies, biological half-life may be increased, and the potential for adverse immune reactions upon administration to humans is reduced.

5

Alternative techniques for generating or selecting antibodies useful herein include *in vitro* exposure of lymphocytes to Zcytor5 protein or peptide, and selection of antibody display libraries in phage or
10 similar vectors (for instance, through use of immobilized or labeled Zcytor5 protein or peptide). Genes encoding polypeptides having potential Zcytor5 polypeptide binding domains can be obtained by screening random peptide libraries displayed on phage (phage display) or on
15 bacteria, such as *E. coli*. Nucleotide sequences encoding the polypeptides can be obtained in a number of ways, such as through random mutagenesis and random polynucleotide synthesis. These random peptide display libraries can be used to screen for peptides which interact with a known
20 target which can be a protein or polypeptide, such as a ligand or receptor, a biological or synthetic macromolecule, or organic or inorganic substances. Techniques for creating and screening such random peptide display libraries are known in the art (Ladner et al., US
25 Patent NO. 5,223,409; Ladner et al., US Patent NO. 4,946,778; Ladner et al., US Patent NO. 5,403,484 and Ladner et al., US Patent NO. 5,571,698) and random peptide display libraries and kits for screening such libraries are available commercially, for instance from Clontech
30 (Palo Alto, CA), Invitrogen Inc. (San Diego, CA), New England Biolabs, Inc. (Beverly, MA) and Pharmacia LKB Biotechnology Inc. (Piscataway, NJ). Random peptide display libraries can be screened using the Zcytor5 sequences disclosed herein to identify proteins which bind
35 to Zcytor5. These "binding proteins" which interact with

Zcytor5 polypeptides can be used for tagging cells; for isolating homolog polypeptides by affinity purification; they can be directly or indirectly conjugated to drugs, toxins, radionuclides and the like. These binding
5 proteins can also be used in analytical methods such as for screening expression libraries and neutralizing activity. The binding proteins can also be used for diagnostic assays for determining circulating levels of polypeptides; for detecting or quantitating soluble
10 polypeptides as marker of underlying pathology or disease. These binding proteins can also act as Zcytor5 "antagonists" to block Zcytor5 binding and signal transduction *in vitro* and *in vivo*.

15 Antibodies can also be generated gene therapy. The animal is administered the DNA or RNA which encodes Zcytor5 or an immunogenic fragment thereof so that cells of the animals are transfected with the nucleic acid and express the protein which in turn elicits an immunogenic
20 response. Antibodies which then are produced by the animal are isolated in the form of polyclonal or monoclonal antibodies.

25 Antibodies to Zcytor5 may be used for tagging cells that express the protein, for affinity purification, within diagnostic assays for determining circulating levels of soluble protein polypeptides, and as antagonists to block ligand binding and signal transduction *in vitro*
30 and *in vivo*.

 Methods for preparing polyclonal and monoclonal antibodies are well known in the art (see for example,
35 Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor, NY (1989); and

Hurrell, J. G. R., Ed., *Monoclonal Hybridoma Antibodies: Techniques and Applications* (CRC Press, Inc., Boca Raton, FL, 1982). As would be evident to one of ordinary skill in the art, polyclonal antibodies can be generated from a variety of warm-blooded animals such as horses, cows, goats, sheep, dogs, chickens, rabbits, mice, and rats. Generally speaking, antibodies against which bind to the claimed Zcytor5 polypeptides can be raised by immunization of animals with a Zcytor5 polypeptide or a fragment thereof. The immunogenicity of a Zcytor5 polypeptide may be increased through the use of an adjuvant such as Freund's complete or incomplete adjuvant. A variety of assays known to those skilled in the art can be utilized to detect antibodies which specifically bind to Zcytor5 polypeptides. Exemplary assays are described in detail in *Antibodies: A Laboratory Manual*, Harlow and Lane (Eds.) (Cold Spring Harbor Laboratory Press, 1988). Representative examples of such assays include: concurrent immunoelectrophoresis, radio-immunoassays, radio-immunoprecipitations, enzyme-linked immunosorbent assays (ELISA), dot blot assays, inhibition or competition assays, and sandwich assays.

Antibodies to Zcytor5 are may be used for tagging cells that express the receptor, for affinity purification, within diagnostic assays for determining circulating levels of soluble receptor polypeptides, and as antagonists to block ligand binding and signal transduction *in vitro* and *in vivo*.

Uses

The tissue specificity of Zcytor5 expression suggests that Zcytor5 may be a receptor for growth and/or maintenance factor in the thyroid heart and skeletal muscle. Zcytor5 could therefor be used to down regulate the effects of the factor by administering soluble Zcytor5 to the patient. For example the soluble receptor could be used to lessen the effect of cardiotrophin-1 on cardiac pathologies. Thus preventing enlargement of the heart due to heart disease. Zcytor5 could also be used as a diagnostic to test for the presence of cardiotrophin-1 in the blood. Furthermore, Zcytor5 can be used to discover other possible ligands which would bind to Zcytor5.

15

The present invention also provides reagents which will find use in diagnostic applications. For example, the Zcytor5 gene. A probe comprising the Zcytor5 DNA or RNA or a subsequence thereof can be used to determine if the Zcytor5 gene is present on chromosome 1 or if a mutation has occurred.

20

Antibodies to Zcytor5 could be used to purify Zcytor5 and as a therapeutic to modulate the effect of the Zcytor5 ligand. The anti-idiotypic antibody to Zcytor5 could be used to purify the ligand of Zcytor5 and the administration of the anti-idiotypic antibody could be used to modulate the effect of the Zcytor5 ligand.

25

The invention is further illustrated by the following non-limiting examples.

30

Example 1Cloning of Human Zcytor5

Human Zcytor5 was identified from expressed
5 sequence tag (EST) 698365 (SEQ ID NO: 7) identified in an
EST database. The cDNA containing EST 698365 was obtained
from Incyte Pharmaceuticals, Inc. as dried DNA. Upon
reconstitution in water, the cDNA was transfected into *E.*
coli strain DH10B. The plasmid was designated pSL8365.
10 The EST in plasmid pSL8365 was sequenced, revealing an
insert of 952 bp.

The GENE TRAPPER® cDNA positive selection system
(Life Technologies, Gaithersburg, MD) employing
15 oligonucleotide ZC11,286 (SEQ ID NO: 8) was used to
isolate the plasmid Hzcytor5-9 from a human lung cDNA
library (obtained from Life Technologies Inc.,
Gaithersburg, MD) in accordance with the manufacturer's
directions. Hzcytor5-9 extended the sequence of pSL8365 by
20 459 bp. The sequence present in Hzcytor5-9 allowed the
isolation of an overlapping EST No. 485212 (SEQ ID NO: 9),
which extended the open reading frame of Hzcytor5-9 by a
further 33 codons.

25 A cDNA encoding full-length Zcytor5 was isolated
from a human testis cDNA library. (See Example 2 for the
preparation of the human cDNA testis library.) The library
was comprised of eighty pools of plasmid DNA, each pool
comprised of 10,000 independent recombinants. The presence
30 of Zcytor5 cDNA in each library pool was determined by PCR
employing primers ZC11,663 (SEQ ID NO: 10) and ZC12,212
(SEQ ID NO: 11). PCR was carried out using AMPLITAQ® DNA
polymerase (Perkin-Elmer) in buffer conditions recommended
by the supplier. The amplification was carried out at 94° C
35 for 1 minute followed by 30 cycles, each cycle consisting
of 20 seconds at 94° C, 1 minute at 66° C and 7 minutes at

74° C. Five cDNA pools were found to be positive for the 420 bp PCR product by agarose gel electrophoresis.

Plasmid DNA from one positive library pool was
5 electrophoresed into DH10B cells and plated. Colony lifts
were prepared using Hybond-N filters (Amersham; Arlington
Heights, IL) according to the procedure provided by the
manufacturer. Following denaturation and neutralization,
DNA was cross-linked onto the filters with 1,200 µJoules
10 of UV energy in a STRATALINKER® (Stratagene Cloning
Systems). Cell debris was removed by several washes in
0.25X standard sodium citrate (SSC), 0.25% sodium dodecyl
sulfate (SDS) and 1 mM EDTA at 65° C. The filters were then
pre-hybridized overnight at 65° C in EXPRESSHYB® solution
15 (Clontech) with 1 mg/ml heat denatured salmon sperm DNA.
Colonies positive for Zcytor5 were identified by
hybridization with a probe that was generated from EST
484212 (SEQ ID NO: 9) cDNA employing PCR primers ZC11,663
(SEQ ID NO: 10) and ZC12,212 (SEQ ID NO: 11). The PCR
20 product probe was purified by agarose gel electrophoresis.
100 ng of the probe was labeled with ³²P dCTP using the
MULTI-PRIME® DNA labeling system (Amersham).
Unincorporated label was removed with a NUCTRAP® column
(Stratagene). Probe hybridization was carried out
25 overnight at 65° C in EXPRESSHYB® solution at a probe
concentration of 1 x10⁶ cpm/ml. The filters were washed at
65° C in a wash buffer containing 0.25X SSC, 0.25 SDS and 1
mM EDTA.

30 Three positive signals were identified and were
subjected to colony purification via a second round of
filter hybridization. Sequence analysis of one positive
clone, SEQ ID NO: 3 was found to be full length human
Zcytor-5. Sequencing of a several overlapping clones
35 revealed a second full-length sequence SEQ ID NO: 1 which
is an allelic variant of SEQ ID NO: 3.

Example 2Construction of the Human Testis cDNA Library

5 Fourteen μ l of poly d(T) - selected poly (A)⁺
human testis mRNA (Clontech) at a concentration of 1.0
 μ g/ μ l was mixed with 2 μ l of 20 pmole/ μ l first strand
primer ZC2938 (SEQ ID NO: 12) containing an Sst I
restriction site. The mixture was heated at 65° C for 4
10 minutes and cooled by chilling on ice. First strand cDNA
synthesis was initiated by the addition of 8 μ l of 250 mM
Tris-HCl, pH 8.3, 375 mM KCl, 15 mM MgCl₂ (5x SUPERScript™
buffer; GIBCO BRL), 4 μ l of 100 mM dithiothreitol (DTT)
and 2 μ l of a deoxynucleotide triphosphate solution
15 containing 10 mM each of dATP, dGTP, dTTP and 5-methyl-
dCTP (Pharmacia LKB Biotechnology Inc.) to the RNA-primer
mixture. The reaction mixture was incubated at 45° C for 4
minutes followed by the addition of 10 μ l of 200 U/ μ l
RNase H⁻reverse transcriptase (GIBCO BRL). The efficiency
20 of the first strand synthesis was analyzed in a parallel
reaction by the addition of 10 μ Ci of ³²P- α dCTP to a 10 μ l
aliquot of the reaction mixture to label the reaction for
analysis. The reactions were incubated at 45° C for 1 hour
followed by an incubation at 50° C for 15 minutes.
25 Unincorporated ³²P- α dCTP in the labeled reaction was
removed by chromatography on a 400 pore size gel
filtration column (CHROMA SPIN + TE-400™; Clontech
Laboratories Inc.). Unincorporated nucleotides in the
unlabeled first strand reaction were removed by twice
30 precipitating the cDNA in the presence of 10 μ g of
glycogen carrier, 2.5 M ammonium acetate and 2.5 volume
ethanol. The unlabeled cDNA was resuspended in 50 μ l water
for use in second strand synthesis. The length of the
labeled first strand cDNA was determined by agarose gel
35 electrophoresis.

Second strand synthesis was performed on first strand cDNA under conditions that promoted first strand priming of second strand synthesis resulting in DNA hairpin formation. The reaction mixture was assembled at room temperature and was comprised of 66 μ l of the unlabeled first strand cDNA, 20 μ l of 5X polymerase I buffer (100 mM Tris: HCl, pH 7.4, 500 mM KCl, 25 mM $MgCl_2$, 50 mM $(NH_4)_2SO_4$), 1 μ l of 100 mM DTT, 1 μ l of a solution containing 20 mM of each deoxynucleotide triphosphate, 3 μ l of 5 mM β -NAD, 1 μ l of 4 U/ μ l of *E. coli* DNA ligase (New England Biolabs Inc., Beverly, MA) and 5 μ l of 10 U/ μ l *E. coli* DNA polymerase I (New England Biolabs, Inc.). The reaction was incubated at room temperature for 5 minutes followed by the addition of 2 μ l of 2.2 U/ μ l RNase H (GIBCO BRL). A parallel reaction in which a 10 μ l aliquot of the second strand synthesis mixture was labeled by the addition of 10 μ Ci ^{32}P - α dCTP was used to monitor the efficiency of second strand synthesis. The reactions were incubated at 15° C for two hours followed by a 15 minute incubation at room temperature. Unincorporated ^{32}P - α dCTP in the labeled reaction was removed by chromatography through a 400 pore size gel filtration column (Clontech Laboratories, Inc.) before analysis by agarose gel electrophoresis. The unlabeled reaction was terminated by two extractions with phenol/chloroform and a chloroform extraction followed by ethanol precipitation in the presence of 2.5 M ammonium acetate.

The single-stranded DNA of the hairpin structure was cleaved using mung bean nuclease. The reaction mixture contained 100 μ l of second strand cDNA, 20 μ l of 10x mung bean nuclease buffer (Stratagene Cloning Systems, La Jolla, CA), 16 μ l of 100 mM DTT, 51.5 μ l of water and 12.5 μ l of a 1:10 dilution of mung bean nuclease (Promega Corp.; final concentration 10.5 U/ μ l) in mung bean nuclease dilution buffer. The reaction was incubated at 37°

C for 15 minutes. The reaction was terminated by the addition of 20 μ l of 1 M Tris: HCl, pH 8.0 followed by sequential phenol/chloroform and chloroform extractions as described above. Following the extractions, the DNA was
5 precipitated in ethanol and resuspended in water.

The resuspended cDNA was blunt-ended with T4 DNA polymerase. The cDNA, which was resuspended in 138 μ l of water, was mixed with 40 μ l of 5X T4 DNA polymerase buffer
10 (250 mM Tris: HCl, pH 8.0, 250 mM KCl, 25 mM $MgCl_2$), 3 μ l 0.1 M DTT, 5 μ l of a solution containing 10 mM of each deoxynucleotide triphosphate and 4 μ l of 1 U/ μ l T4 DNA polymerase (Boehringer Mannheim Corp., Indianapolis, IN). After incubation of 1 hour at 10° C, the reaction was
15 terminated by the addition of 10 μ l of 0.5 M EDTA followed by serial phenol/chloroform and chloroform extractions as described above. The DNA was chromatographed through a 400 pore size gel filtration column (Clontech Laboratories Inc. Palo Alto, CA) to
20 remove trace levels of protein and to remove short cDNAs less than about 400 bp in length. The DNA was ethanol precipitated in the presence of 12 μ g glycogen carrier and 2.5 M ammonium acetate and was resuspended in 10 μ l of water. Based on the incorporation of ^{32}P - α dCTP, the yield
25 of cDNA was estimated to be about 2 μ g from a starting template of 12.5 μ g.

Eco RI adapters were ligated onto the 5' ends of the cDNA to enable cloning into a lambda phage vector. A
30 10 μ l aliquot of cDNA (containing about 2 μ g of cDNA) and 11 μ l of 65 pmole/ μ l of *Eco* RI adapter (Pharmacia LKB Biotechnology Inc.) were mixed with 3 μ l 10x ligase buffer (Promega Corp.), 3 μ l 10 mM ATP and 3 μ l of 15 U/ μ l T4 DNA ligase (Promega Corp.). The reaction was incubated
35 overnight (about 18 hours) at 12.5° C. The reaction was terminated by the addition of 150 μ l of water and 10 μ l of

3 M Na acetate, followed by incubation at 65° C for 30 minutes. After incubation, the cDNA was extracted with phenol/chloroform and chloroform as described above and precipitated in the presence of 2.5 M ammonium acetate and
5 1.2 volume of isopropanol. Following centrifugation, the cDNA pellet was washed with 70% ethanol, air dried and resuspended in 89 µl water.

To facilitate the directional cloning of the
10 cDNA into a lambda phage vector, the cDNA was digested with *Sst*-I resulting in a cDNA having 5' *Eco* RI and 3' *Sst*-I cohesive ends. The *Sst*-I restriction site at the 3' end of the cDNA had been previously introduced through primer ZC2938 (SEQ ID NO: 12). Restriction enzyme
15 digestion was carried out in a reaction containing 89 µl of cDNA described above, 10 µl of 6 mM Tris: HCl, 6 mM MgCl₂, 150 mM NaCl, 1 mM DTT (10x D buffer; Promega Corp., Madison, WI) and 1 µl of 12 U/µl Not I (Promega Corp.). Digestion was carried out at 37° C for 1 hour. The reaction
20 was terminated by serial phenol/chloroform and chloroform extractions. The cDNA was ethanol precipitated, washed with 70% ethanol, air dried and resuspended in 20 µl of 1x gel loading buffer (10 mM Tris: HCl, pH 8.0, 1 mM EDTA, 5% glycerol and 0.125% bromphenol blue).

25 The resuspended cDNA was heated to 65° C for 5 minutes, cooled on ice and electrophoresed on a 0.8% low melt agarose gel (SEA PLAQUE GTG™ low melt agarose; FMC Corp.). Unincorporated adapters and cDNA below 1.6 kb in
30 length were excised from the gel. The electrodes were reversed, and the cDNA was electrophoresed until concentrated near the lane of origin. The area of the gel containing the concentrated cDNA was excised and placed in a microfuge tube, and the approximate volume of the gel
35 slice was determined. A 300 µl aliquot of water, approximately three times the volume of the gel slice, was

added to the tube. The agarose was then melted by heating to 65° C for 15 minutes. Following equilibration of the sample to 42° C, 10 µl of 1 U/µl β-agarose I (New England Biolabs, Inc.) was added, and the mixture was incubated
5 for 90 minutes to digest the agarose. After incubation, 40 µl of 3 M Na acetate was added to the sample, and the mixture was incubated on ice for 15 minutes. The sample was centrifuged at 14,000 x g for 15 minutes at room temperature to remove undigested agarose. The cDNA in the
10 supernatant was ethanol precipitated, washed in 70% ethanol, air-dried and resuspended in 37 µl of water for the kinase reaction to phosphorylate the ligated Eco RI adapters.

15 To the 37 µl cDNA solution described above was added 10 µl of 10x ligase buffer (Stratagene Cloning Systems), and the mixture was heated to 65° C for 5 minutes. The mixture was cooled on ice, and 5 µl of 10 mM ATP and 3 µl of 10 U/µl of T4 polynucleotide kinase
20 (Stratagene Cloning Systems) were added. The reaction was incubated at 37° C for 45 minutes and was terminated by heating to 65° C for 10 minutes followed by serial extractions with phenol/chloroform and chloroform. The phosphorylated cDNA was ethanol precipitated in the
25 presence of 2.5 M ammonium acetate, washed with 70% ethanol, air dried and resuspended in 12.5 µl water. The concentration of the phosphorylated cDNA was estimated to be about 40 fmole/µl.

Example 3Northern Blot Analysis of Human Zcytor5

A 300bp double stranded DNA probe for Northern
5 analysis was prepared from pSL1034 by PCR using
oligonucleotide primers ZC 10,787 (SEQ ID NO:13) and ZC
11,097 (SEQ ID NO:14). The 300 bp PCR fragment was gel-
purified using a QIAQUICK® purification kit (Qiagen Inc.,
Chatsworth, CA) and random-primer labeled using a
10 MULTIPRIME® kit (Amersham Corp.). Labeled cDNA was
purified from free counts using a Stratagene push column.
Human multiple tissue Northern blots (Clontech
Laboratories) and a human fetal tissue Northern blot
(Clontech Laboratories) were pre-hybridized for three
15 hours at 68°C using EXPRESSHYB hybridization solution
(Clontech Laboratories). The ³²P-labeled cDNA probe was
then added to 10 mls of fresh hybridization solution at
10⁶cpm/ml overnight at 68°C. The blots were washed
several times at room temperature in wash solution
20 containing 2X SSC, 0.05% SDS, then with continuous
agitation for 40 min at room temperature. The blots were
then washed in 0.1X SSC, 0.1% SDS at 50°C for 40 min with
one change of wash solution.

25 A single transcript of ~2.3 kb was detected
after exposure to film. In the multiple tissue blots (MTN,
MTN II and MTN III; Clontech Laboratories) the transcript
was present in highest abundance in placenta, thyroid,
heart and skeletal muscle with lower levels in prostate
30 and trachea. Trace mRNA levels were found in kidney,
pancreas, testis, small intestine, colon, lymph node,
adrenal cortex and bone marrow.

Example 4Chromosomal Assignment and Placement of Human Zcytor-5

Zcytor5 was mapped to chromosome 19 using the
5 commercially available version of the "Stanford G3
Radiation Hybrid Mapping Panel" (Research Genetics, Inc.,
Huntsville, AL). The "Stanford G3 RH Panel" contains
PCRable DNAs from each of 83 radiation hybrid clones of
the whole human genome, plus two control DNAs (the RM
10 donor and the A3 recipient). A publicly available WWW
server (<http://shgc-www.stanford.edu>) allows chromosomal
localization of markers.

For the mapping of Zcytor5 with the "Stanford G3 RH
15 Panel", 20 μ l reactions were set up in a PCRable 96-well
microtiter plate (Stratagene, La Jolla, CA) and used in a
"RoboCycler Gradient 96" thermal cycler (Stratagene). Each
of the 85 PCR reactions consisted of 2 μ l 10X KlenTaq PCR
reaction buffer (CLONTECH Laboratories, Inc., Palo Alto,
20 CA), 1.6 μ l dNTPs mix (2.5 mM each, PERKIN-ELMER, Foster
City, CA), 1 μ l sense primer, (SEQ ID NO:13) 5' TAT GGC
CAG GAC AAC ACA 3', 1 μ l antisense primer, (SEQ ID NO:14),
5' ATA GGG CGT AAA GAG AGC 3', 2 μ l "RediLoad" (Research
Genetics, Inc., Huntsville, AL), 0.4 μ l 50X Advantage
25 KlenTaq Polymerase Mix (Clontech Laboratories, Inc.), 25
ng of DNA from an individual hybrid clone or control and x
 μ l ddH₂O for a total volume of 20 μ l. The reactions were
overlaid with an equal amount of mineral oil and sealed.
The PCR cycler conditions were as follows: an initial 1
30 cycle 5 minute denaturation at 95°C, 35 cycles of a 1
minute denaturation at 95°C, 1 minute annealing at 66°C and
1.5 minute extension at 72°C, followed by a final 1 cycle
extension of 7 minutes at 72°C. The reactions were
separated by electrophoresis on a 2% agarose gel (Life
35 Technologies, Gaithersburg, MD).

The results showed linkage of Zcytor5 to the framework marker WI-7289 with a LOD score of >10 and at a distance of 14.67cR_10000 from the marker. The use of surrounding markers positions Zcytor5 in the 19p13.1-p11
5 region on the integrated LDB chromosome 19 map (The Genetic Location Database, University of Southampton, WWW server: http://cedar.genetics.soton.ac.uk/public_html/) .

Example 5

10 Cloning of the Rat Zcytor5 Gene

Rat Zcytor5 cDNA encoding Zcytor5 was isolated from an amplified Rat testis cDNA library with a probe that was generated by primers ZC12212 (SEQ ID NO:
15 11) and ZC10785 (SEQ ID NO:15) and 10 ng of plasmid pSL85212 as a template obtained from cDNA containing EST 698365 as described in Example 1. The probe was prepared by PCR by combining 1 µl containing 10 ng of pSL85212, 1
20 µl of ZC12212 having a concentration of 20 pmole/µl, 1µl of ZC10785 having a concentration of 20 pmole/µl, 0.5µl of dNTP having a concentration of 20 mM of dATP, dGTP, dCTP and dTTP, 5 µl of 10X Klentaq polymerase buffer (Clontech) 5µl Klentaq DNA polymerase (Clontech) and 39.5µl water. The amplification was carried out at 94° C for 1 minute
25 followed by 30 cycles, each cycle consisting of 15 seconds at 95° C, 20 seconds at 62° C and 1 minutes at 68° C. The reaction had a final incubation at 68° C for 10 minutes.

The resulting PCR product was diluted 1:100 with
30 water. Four µl of the diluted PCR product was re-amplified using the above-described conditions and the resultant PCR product was further purified by electrophoresis on low-melt agarose gel. The DNA probe was recovered from low-melt gel by digestion with β-Agarose I digestion. The rat
35 Zcytor5 gene was then cloned from a rat testis library which was constructed as described below in Example 6.

In cloning the rat Zcytor5 gene, the library was first amplified by plating 3×10^6 plaque forming units (pfu) from the previously constructed primary library onto 98
5 150 mm NZY plates. Ten ml of serum medium was added to each plate and was incubated for several hours at room temperature. Following incubation, the phage lysates were collected and pooled to yield the amplified phage library.

10 1.5 million pfus from the amplified rat testis cDNA library were plated onto 150 mm NZY plates at a density of 40,000 pfu/plate on XL-1 Blue MRF' host cells. Following incubation at 37° C overnight, filter lifts were made using HYBOND-NTM membranes (Amersham), according to
15 the procedures provided by the manufacturer. The filters were processed by denaturation in solution containing 1.5 M NaCl and 0.5 M NaOH for 8 minutes at room temperature. The filters were neutralized in 0.5 M Tris: HCl, pH 7.2 for 5 minutes. Phage DNA was fixed onto the filters with
20 1,200 µJoules of UV energy in a UV Cross-linker (Stratagene). The filters were then washed with 0.25X SSC at 70° C to remove excess cellular debris. Filter pre-hybridization was carried out in a hybridization solution containing 5X SSC, 5X Denhardt solution, 0.2% SDS, 1 mM
25 EDTA and heat denatured sheared salmon-sperm DNA at a final concentration of 100 µg/ml for 72 hours at 60° C.

75 ng of probe DNA was labeled with ³²P-dCTP using a MEGAPRIME® labeling kit (Amersham) and was
30 purified with a NUCTRAP® column (Stratagene). The labeled probe was heat-denatured and added to fresh hybridization solution at a concentration of 1.5×10^6 cpm/ml. Into this solution were also added the filters containing the phage particles. Hybridization of the probes to the phage-
35 containing filters was completed overnight at 45° C. Following hybridization, the filters were washed in a

solution containing 0.25X SSC, 0.25% SDS and 1 mM EDTA at 50° C. The washed filters were autoradiographed for 72 hours at -70° C with intensifying screens. Examination of the autoradiographs revealed multiple regions that
5 hybridized with the labeled probe. Agar plugs were picked from 56 regions for plaque purification. Of the positive signals, eleven produce positive phagemids following secondary and tertiary hybridization screens. The plasmids within the positive phagemids were recovered using the
10 EXASSIT/SOLR™ system according to the manufacturer's specifications. A clone designated pSLRatR5-1 was sequenced and found to encode full length Rat Zcytor5 (SEQ ID NO: 5)

15

Example 6

Production of Rat Testis cDNA library

The rat first strand cDNA reaction contained 10
20 µl of rat testis poly d(T)-selected poly (A)⁺ mRNA (Clontech, Palo Alto, CA) at a concentration of 1.0 µg/µl, and 2 µl of 20 pmole/µl first strand primer ZC6091 (SEQ ID NO: 16) containing an Xho I restriction site. The mixture was heated at 70°C for 4 minutes and cooled by chilling on
25 ice. First strand cDNA synthesis was initiated by the addition of 8 µl of first strand buffer (5x SUPERScript™ buffer; Life Technologies, Gaithersburg, MD), 4 µl of 100 mM dithiothreitol, and 2 µl of a deoxynucleotide triphosphate solution containing 10 mM each of dATP, dGTP
30 and 5-methyl-dCTP (Pharmacia LKB Biotechnology, Piscataway, NJ) to the RNA-primer mixture. The reaction mixture was incubated at 45° C for 2 minutes, followed by the addition of 10 µl of 200 U/µl RNase H- reverse transcriptase (SUPERScript II®; Life Technologies). The
35 efficiency of the first strand synthesis was analyzed in a parallel reaction by the addition of 10 µCi of ³²P-αdCTP

to 5 μ l aliquot from one of the reaction mixtures to label the reaction for analysis. The reactions were incubated at 45°C for 1 hour followed by an incubation at 50° C for 10 minutes. Unincorporated 32 P- α dCTP in the labeled
5 reaction was removed by chromatography on a 400 pore size gel filtration column (Clontech). The unincorporated nucleotides and primers in the unlabeled first strand retains were removed by chromatography on 400 pore size gel filtration column (Clontech). The length of labeled
10 first strand cDNA was determined by agarose gel electrophoresis.

The second strand reaction contained 102 μ l of the unlabeled first strand cDNA, 30 μ l of 5x polymerase I
15 buffer (125 mM Tris: HCl, pH 7.5, 500 mM KCl, 25 mM MgCl_2 , 50mM $(\text{NH}_4)_2\text{SO}_4$), 2 μ l of 100 mM dithiothreitol, 3 μ l of a solution containing 10 mM of each deoxynucleotide triphosphate, 5 μ l of 5 mM β -NAD, 2 μ l of 3 U/ μ l *E. coli* DNA ligase (New England Biolabs), 5 μ l of 10 U/ μ l *E. coli*
20 DNA polymerase I (New England Biolabs), and 1.5 μ l of 2 U/ μ l RNase H (Life Technologies). A 10 μ l aliquot from one of the second strand synthesis reactions was labeled by the addition of 10 μ Ci 32 P- α dCTP to monitor the efficiency of second strand synthesis. The reactions were incubated
25 at 16° C for two hours, followed by the addition of 10 μ l T4 DNA polymerase (10 U/ μ l, Boehringer Mannheim) and incubated for an additional 5 minutes at 16°C. Unincorporated 32 P- α dCTP in the labeled reaction was removed by chromatography through a 400 pore size gel
30 filtration column (Clontech) before analysis by agarose gel electrophoresis. The unlabeled was terminated by the addition of 20 μ l 0.5 EDTA and extraction with phenol/chloroform and chloroform followed by ethanol precipitation in the presence of 2.5 M ammonium acetate.
35 The yield of cDNA was estimated to be approximately 2 μ g from starting mRNA template of 10 μ g.

Eco RI adapters were ligated onto the 5' ends of the cDNA described above to enable cloning into an expression vector. A 10.5 µl aliquot of cDNA (~2 µg) and 5 µl of 65 pmole/µl of *Eco* RI adapter (Pharmacia LKB Biotechnology Inc.) were mixed with 2.5 µl 10x ligase buffer 66 mM Tris-HCl pH 7.5, 10 mM MgCl₂, 2.5 µl of 10 mM ATP and 1 µl of 15 U/µl T4 DNA ligase (Promega Corp., Madison, WI). The reaction was incubated overnight (~12 hours) at 12°C. The reaction was terminated by incubation at 70°C for 20 minutes. After incubation, the reaction was cooled to 37°C. To the reaction was added 2.5 µl 10mM ATP and 3 µl 10 U/µl T4 polynucleotide kinase (Life Technologies) to phosphorylate the ligated *Eco* RI adapters.

To facilitate the directional cloning of the cDNA into an expression vector, the cDNA was digested with *Xho* I, resulting in a cDNA having a 5' *Eco* RI cohesive end and a 3' *Xho* I cohesive end. The *Xho* I restriction site at the 3' end of the cDNA had been previously introduced using the ZC6091 primer (SEQ ID NO: 3). Restriction enzyme digestion was carried out in a reaction mixture containing 25 µl of cDNA described above, 15 µl of 10x H Buffer (Boehringer Mannheim), 109 µl H₂O, and 1.0 µl of 40 U/µl *Xho* I (Boehringer Mannheim). Digestion was carried out at 37°C for 40 minutes. The reaction was terminated by incubation at 65° C for 10 minutes and chromatography through a 400 pore size gel filtration column (Clontech).

The cDNA was ethanol precipitated, washed with 70% ethanol, air dried and resuspended in 20 µl of 1x gel loading buffer (10 mM Tris:HCl, pH 8.0, 1 mM EDTA, 5% glycerol and 0.125% bromphenol blue). The resuspended cDNA was heated to 65° C for 5 minutes, cooled on ice and electrophoresed on a 0.8% low melt agarose gel. The

contaminating adapters and cDNA below 0.6 Kb in length were excised from the gel. The electrodes were reversed, and the cDNA was electrophoresed until concentrated near the lane origin. The area of the gel containing the
5 concentrated cDNA was excised and placed in a microfuge tube, and the approximate volume of the gel slice was determined. An aliquot of water approximately three times the volume of the gel slice (300 μ l) and 35 μ l 10x β -agarose I buffer (New England Biolabs) was added to the
10 tube, and the agarose was melted by heating to 65°C for 15 minutes. Following equilibration of the sample to 45°C, 3 μ l of 1 U/ μ l β -agarose I (New England Biolabs) was added, and the mixture was incubated for 60 minutes at 45°C to digest the agarose. After incubation, 40 μ l of 3 M Na
15 acetate was added to the sample, and the mixture was incubated on ice for 15 minutes. The sample was centrifuged at 14,000 x g for 15 minutes at room temperature to remove undigested agarose. The cDNA was ethanol precipitated, washed in 70% ethanol, air-dried and
20 resuspended in 10 μ l water.

The resulting cDNA was cloned into the lambda phage vector λ ZapII that was predigested with Eco RI and Xho I and dephosphorylated (Stratagene Cloning Systems, La
25 Jolla, CA). Ligation of the cDNA to the λ ZapII vector was carried out in a reaction mixture containing 1.0 μ l of prepared vector, 1.0 μ l of rat testis cDNA, 1.0 μ l 10X Ligase Buffer (Promega), 1.0 μ l of 10 mM ATP, 5 μ l water, and 1.0 μ l of T4 DNA Ligase at 15 units/ml (Promega). The
30 ligation mixture was incubated at 5°C-15°C overnight in a temperature gradient. After incubation, the ligation mixture was packaged into phage using GIGPACK III GOLD packaging extract (Stratagene Cloning Systems) and the resulting library was titered according to the
35 manufacturer's specifications.

SEQUENCE LISTING

(1) GENERAL INFORMATION

- (i) APPLICANT: ZymoGenetics, Inc.
1201 Eastlake Avenue East
Seattle
WA
USA
98102
- (ii) TITLE OF THE INVENTION: MAMMALIAN ZCYTOR5
- (iii) NUMBER OF SEQUENCES: 37
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Zymogenetics
 - (B) STREET: 1201 Eastlake Ave East
 - (C) CITY: Seattle
 - (D) STATE: WA
 - (E) COUNTRY: USA
 - (F) ZIP: 98102
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Diskette
 - (B) COMPUTER: IBM Compatible
 - (C) OPERATING SYSTEM: DOS
 - (D) SOFTWARE: FastSEQ for Windows Version 2.0
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Lunn, Paul G
 - (B) REGISTRATION NUMBER: 32,743

(C) REFERENCE/DOCKET NUMBER: 96-22PC

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 206-442-6627

(B) TELEFAX: 206-442-6678

(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1690 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 52...1317

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CGCCACGCGC CGAGCCGCAG CCCGCCGCGC GCCCCGGCA GCGCCGGCCC C ATG CCC	57
Met Pro	
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GCC GGC CGC CGG GGC CCC GCC GCC CAA TCC GCG CGG CGG CCG CCG CCG	105
Ala Gly Arg Arg Gly Pro Ala Ala Gln Ser Ala Arg Arg Pro Pro Pro	
5 10 15	
TTG CTG CCC CTG CTG CTG CTG CTC TGC GTC CTC GGG GCG CCG CGA GCC	153
Leu Leu Pro Leu Leu Leu Leu Leu Cys Val Leu Gly Ala Pro Arg Ala	
20 25 30	
GGA TCA GGA GCC CAC ACA GCT GTG ATC AGT CCC CAG GAT CCC ACG CTT	201
Gly Ser Gly Ala His Thr Ala Val Ile Ser Pro Gln Asp Pro Thr Leu	
35 40 45 50	
CTC ATC GGC TCC TCC CTG CTG GCC ACC TGC TCA GTG CAC GGA GAC CCA	249
Leu Ile Gly Ser Ser Leu Leu Ala Thr Cys Ser Val His Gly Asp Pro	
55 60 65	

CCA	GGA	GCC	ACC	GCC	GAG	GGC	CTC	TAC	TGG	ACC	CTC	AAT	GGG	CGC	CGC	297
Pro	Gly	Ala	Thr	Ala	Glu	Gly	Leu	Tyr	Trp	Thr	Leu	Asn	Gly	Arg	Arg	
			70					75					80			
CTG	CCC	CCT	GAG	CTC	TCC	CGT	GTA	CTC	AAC	GCC	TCC	ACC	TTG	GCT	CTG	345
Leu	Pro	Pro	Glu	Leu	Ser	Arg	Val	Leu	Asn	Ala	Ser	Thr	Leu	Ala	Leu	
		85					90					95				
GCC	CTG	GCC	AAC	CTC	AAT	GGG	TCC	AGG	CAG	CGG	TCG	GGG	GAC	AAC	CTC	393
Ala	Leu	Ala	Asn	Leu	Asn	Gly	Ser	Arg	Gln	Arg	Ser	Gly	Asp	Asn	Leu	
	100					105					110					
GTG	TGC	CAC	GCC	CGT	GAC	GGC	AGC	ATC	CTG	GCT	GGC	TCC	TGC	CTC	TAT	441
Val	Cys	His	Ala	Arg	Asp	Gly	Ser	Ile	Leu	Ala	Gly	Ser	Cys	Leu	Tyr	
	115				120					125					130	
GTT	GGC	CTG	CCC	CCA	GAG	AAA	CCC	GTC	AAC	ATC	AGC	TGC	TGG	TCC	AAG	489
Val	Gly	Leu	Pro	Pro	Glu	Lys	Pro	Val	Asn	Ile	Ser	Cys	Trp	Ser	Lys	
				135					140					145		
AAC	ATG	AAG	GAC	TTG	ACC	TGC	CGC	TGG	ACG	CCA	GGG	GCC	CAC	GGG	GAG	537
Asn	Met	Lys	Asp	Leu	Thr	Cys	Arg	Trp	Thr	Pro	Gly	Ala	His	Gly	Glu	
			150					155					160			
ACC	TTC	CTC	CAC	ACC	AAC	TAC	TCC	CTC	AAG	TAC	AAG	CTT	AGG	TGG	TAT	585
Thr	Phe	Leu	His	Thr	Asn	Tyr	Ser	Leu	Lys	Tyr	Lys	Leu	Arg	Trp	Tyr	
		165					170					175				
GGC	CAG	GAC	AAC	ACA	TGT	GAG	GAG	TAC	CAC	ACA	GTG	GGG	CCC	CAC	TCC	633
Gly	Gln	Asp	Asn	Thr	Cys	Glu	Glu	Tyr	His	Thr	Val	Gly	Pro	His	Ser	
	180					185					190					
TGC	CAC	ATC	CCC	AAG	GAC	CTG	GCT	CTC	TTT	ACG	CCC	TAT	GAG	ATC	TGG	681
Cys	His	Ile	Pro	Lys	Asp	Leu	Ala	Leu	Phe	Thr	Pro	Tyr	Glu	Ile	Trp	
	195				200					205					210	
GTG	GAG	GCC	ACC	AAC	CGC	CTG	GGC	TCT	GCC	CGC	TCC	GAT	GTA	CTC	ACG	729
Val	Glu	Ala	Thr	Asn	Arg	Leu	Gly	Ser	Ala	Arg	Ser	Asp	Val	Leu	Thr	
				215					220					225		
CTG	GAT	ATC	CTG	GAT	GTG	GTG	ACC	ACG	GAC	CCC	CCG	CCC	GAA	GTG	CAC	777
Leu	Asp	Ile	Leu	Asp	Val	Val	Thr	Thr	Asp	Pro	Pro	Pro	Glu	Val	His	
			230					235					240			

65

GTG	AGC	CGC	GTC	GGG	GGC	CTG	GAG	GAC	CAG	CTG	AGC	GTG	CGC	TGG	GTG	825
Val	Ser	Arg	Val	Gly	Gly	Leu	Glu	Asp	Gln	Leu	Ser	Val	Arg	Trp	Val	
		245					250					255				
TCG	CCA	CCC	GCC	CTC	AAG	GAT	TTC	CTC	TTT	CAA	GCC	AAA	TAC	CAG	ATC	873
Ser	Pro	Pro	Ala	Leu	Lys	Asp	Phe	Leu	Phe	Gln	Ala	Lys	Tyr	Gln	Ile	
	260					265				270						
CGC	TAC	CGA	GTG	GAG	GAC	AGT	GTG	GAC	TGG	AAG	GTG	GTG	GAC	GAT	GTG	921
Arg	Tyr	Arg	Val	Glu	Asp	Ser	Val	Asp	Trp	Lys	Val	Val	Asp	Asp	Val	
275					280				285						290	
AGC	AAC	CAG	ACC	TCC	TGC	CGC	CTG	GCC	GGC	CTG	AAA	CCC	GGC	ACC	GTG	969
Ser	Asn	Gln	Thr	Ser	Cys	Arg	Leu	Ala	Gly	Leu	Lys	Pro	Gly	Thr	Val	
			295						300					305		
TAC	TTC	GTG	CAA	GTG	CGC	TGC	AAC	CCC	TTT	GGC	ATC	TAT	GGC	TCC	AAG	1017
Tyr	Phe	Val	Gln	Val	Arg	Cys	Asn	Pro	Phe	Gly	Ile	Tyr	Gly	Ser	Lys	
		310						315					320			
AAA	GCC	GGG	ATC	TGG	AGT	GAG	TGG	AGC	CAC	CCC	ACA	GCC	GCC	TCC	ACT	1065
Lys	Ala	Gly	Ile	Trp	Ser	Glu	Trp	Ser	His	Pro	Thr	Ala	Ala	Ser	Thr	
		325					330					335				
CCC	CGC	AGT	GAG	CGC	CCG	GGC	CCG	GGC	GGC	GGG	GCG	TGC	GAA	CCG	CGG	1113
Pro	Arg	Ser	Glu	Arg	Pro	Gly	Pro	Gly	Gly	Gly	Ala	Cys	Glu	Pro	Arg	
	340					345					350					
GGC	GGA	GAG	CCG	AGC	TCG	GGG	CCG	GTG	CGG	CGC	GAG	CTC	AAG	CAG	TTC	1161
Gly	Gly	Glu	Pro	Ser	Ser	Gly	Pro	Val	Arg	Arg	Glu	Leu	Lys	Gln	Phe	
355					360					365					370	
CTG	GGC	TGG	CTC	AAG	AAG	CAC	GCG	TAC	TGC	TCC	AAC	CTC	AGC	TTC	CGC	1209
Leu	Gly	Trp	Leu	Lys	Lys	His	Ala	Tyr	Cys	Ser	Asn	Leu	Ser	Phe	Arg	
			375						380					385		
CTC	TAC	GAC	CAG	TGG	CGA	GCC	TGG	ATG	CAG	AAG	TCG	CAC	AAG	ACC	CGC	1257
Leu	Tyr	Asp	Gln	Trp	Arg	Ala	Trp	Met	Gln	Lys	Ser	His	Lys	Thr	Arg	
		390						395					400			
AAC	CAG	GAC	GAG	GGG	ATC	CTG	CCC	TCG	GGC	AGA	CGG	GGC	ACG	GCG	AGA	1305
Asn	Gln	Asp	Glu	Gly	Ile	Leu	Pro	Ser	Gly	Arg	Arg	Gly	Thr	Ala	Arg	
		405					410					415				

GGT CCT GCC AGA TAAGCTGTAG GGGCTCAGGC CACCCTCCCT GCCACGTGGA GACGC 1362
 Gly Pro Ala Arg
 420

AGAGGCCGAA CCCAACTGG GGCCACCTCT GTACCCTCAC TTCAGGGCAC CTGAGCCACC 1422
 CTCAGCAAGA GCTGGGGTGG CCCCTGAGCT CCAACGGCCA TAACAGCTCT GACTCCCACG 1482
 TGAGGCCACC TTTGGGTGCA CCCAGTGGG TGTGTGTGTG TGTGTGAGGG TTGGTTGAGT 1542
 TGCCTAGAAC CCCTGCCAGG GCTGGGGGTG AGAAGGGGAG TCATTACTCC CCATTACCTA 1602
 GGGCCCCCTCC AAAAGAGTCC TTTTAAATAA ATGAGCTATT TAGGTGCAAA AAAAAAAAAA 1662
 AAAAAAAAAAT TGCCCTCGTG CCGAATTC 1690

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 422 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Pro	Ala	Gly	Arg	Arg	Gly	Pro	Ala	Ala	Gln	Ser	Ala	Arg	Arg	Pro
1				5					10					15	
Pro	Pro	Leu	Leu	Pro	Leu	Leu	Leu	Leu	Leu	Cys	Val	Leu	Gly	Ala	Pro
			20					25						30	
Arg	Ala	Gly	Ser	Gly	Ala	His	Thr	Ala	Val	Ile	Ser	Pro	Gln	Asp	Pro
		35					40					45			
Thr	Leu	Leu	Ile	Gly	Ser	Ser	Leu	Leu	Ala	Thr	Cys	Ser	Val	His	Gly
	50					55					60				
Asp	Pro	Pro	Gly	Ala	Thr	Ala	Glu	Gly	Leu	Tyr	Trp	Thr	Leu	Asn	Gly
65					70				75					80	
Arg	Arg	Leu	Pro	Pro	Glu	Leu	Ser	Arg	Val	Leu	Asn	Ala	Ser	Thr	Leu
				85					90					95	
Ala	Leu	Ala	Leu	Ala	Asn	Leu	Asn	Gly	Ser	Arg	Gln	Arg	Ser	Gly	Asp
			100					105						110	
Asn	Leu	Val	Cys	His	Ala	Arg	Asp	Gly	Ser	Ile	Leu	Ala	Gly	Ser	Cys
		115					120					125			
Leu	Tyr	Val	Gly	Leu	Pro	Pro	Glu	Lys	Pro	Val	Asn	Ile	Ser	Cys	Trp
	130					135					140				
Ser	Lys	Asn	Met	Lys	Asp	Leu	Thr	Cys	Arg	Trp	Thr	Pro	Gly	Ala	His
145					150					155					160

Gly Glu Thr Phe Leu His Thr Asn Tyr Ser Leu Lys Tyr Lys Leu Arg
 165 170 175
 Trp Tyr Gly Gln Asp Asn Thr Cys Glu Glu Tyr His Thr Val Gly Pro
 180 185 190
 His Ser Cys His Ile Pro Lys Asp Leu Ala Leu Phe Thr Pro Tyr Glu
 195 200 205
 Ile Trp Val Glu Ala Thr Asn Arg Leu Gly Ser Ala Arg Ser Asp Val
 210 215 220
 Leu Thr Leu Asp Ile Leu Asp Val Val Thr Thr Asp Pro Pro Pro Glu
 225 230 235 240
 Val His Val Ser Arg Val Gly Gly Leu Glu Asp Gln Leu Ser Val Arg
 245 250 255
 Trp Val Ser Pro Pro Ala Leu Lys Asp Phe Leu Phe Gln Ala Lys Tyr
 260 265 270
 Gln Ile Arg Tyr Arg Val Glu Asp Ser Val Asp Trp Lys Val Val Asp
 275 280 285
 Asp Val Ser Asn Gln Thr Ser Cys Arg Leu Ala Gly Leu Lys Pro Gly
 290 295 300
 Thr Val Tyr Phe Val Gln Val Arg Cys Asn Pro Phe Gly Ile Tyr Gly
 305 310 315 320
 Ser Lys Lys Ala Gly Ile Trp Ser Glu Trp Ser His Pro Thr Ala Ala
 325 330 335
 Ser Thr Pro Arg Ser Glu Arg Pro Gly Pro Gly Gly Gly Ala Cys Glu
 340 345 350
 Pro Arg Gly Gly Glu Pro Ser Ser Gly Pro Val Arg Arg Glu Leu Lys
 355 360 365
 Gln Phe Leu Gly Trp Leu Lys Lys His Ala Tyr Cys Ser Asn Leu Ser
 370 375 380
 Phe Arg Leu Tyr Asp Gln Trp Arg Ala Trp Met Gln Lys Ser His Lys
 385 390 395 400
 Thr Arg Asn Gln Asp Glu Gly Ile Leu Pro Ser Gly Arg Arg Gly Thr
 405 410 415
 Ala Arg Gly Pro Ala Arg
 420

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1813 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 88...1362

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AATTCGGCAC GAGGGGCTG CGTCCCGCGC CGTGCGCCAC CGCCGCCGAG CCGCAGCCCG	60
CCGCGCGCCC CCGGCAGCGC CGGCCCC ATG CCC GCC GGC CGC CGG GGC CCC GCC	114
Met Pro Ala Gly Arg Arg Gly Pro Ala	
1 5	
GCC CAA TCC GCG CGG CGG CCG CCG CCG TTG CTG CCC CTG CTG CTG CTC	162
Ala Gln Ser Ala Arg Arg Pro Pro Pro Leu Leu Pro Leu Leu Leu	
10 15 20 25	
TGC GTC CTC GGG GCG CCG CGA GCC GGA TCA GGA GCC CAC ACA GCT GTG	210
Cys Val Leu Gly Ala Pro Arg Ala Gly Ser Gly Ala His Thr Ala Val	
30 35 40	
ATC AGT CCC CAG GAT CCC ACG CTT CTC ATC GGC TCC TCC CTG CTG GCC	258
Ile Ser Pro Gln Asp Pro Thr Leu Leu Ile Gly Ser Ser Leu Leu Ala	
45 50 55	
ACC TGC TCA GTG CAC GGA GAC CCA CCA GGA GCC ACC GCC GAG GGC CTC	306
Thr Cys Ser Val His Gly Asp Pro Pro Gly Ala Thr Ala Glu Gly Leu	
60 65 70	
TAC TGG ACC CTC AAT GGG CGC CGC CTG CCC CCT GAG CTC TCC CGT GTA	354
Tyr Trp Thr Leu Asn Gly Arg Arg Leu Pro Pro Glu Leu Ser Arg Val	
75 80 85	
CTC AAC GCC TCC ACC TTG GCT CTG GCC CTG GCC AAC CTC AAT GGG TCC	402
Leu Asn Ala Ser Thr Leu Ala Leu Ala Leu Ala Asn Leu Asn Gly Ser	
90 95 100 105	
AGG CAG CGG TCG GGG GAC AAC CTC GTG TGC CAC GCC CGT GAC GGC AGC	450
Arg Gln Arg Ser Gly Asp Asn Leu Val Cys His Ala Arg Asp Gly Ser	
110 115 120	
ATC CTG GCT GGC TCC TGC CTC TAT GTT GGC CTG CCC CCA GAG AAA CCC	498
Ile Leu Ala Gly Ser Cys Leu Tyr Val Gly Leu Pro Pro Glu Lys Pro	
125 130 135	

GTC	AAC	ATC	AGC	TGC	TGG	TCC	AAG	AAC	ATG	AAG	GAC	TTG	ACC	TGC	CGC	546
Val	Asn	Ile	Ser	Cys	Trp	Ser	Lys	Asn	Met	Lys	Asp	Leu	Thr	Cys	Arg	
		140					145					150				
TGG	ACG	CCA	GGG	GCC	CAC	GGG	GAG	ACC	TTC	CTC	CAC	ACC	AAC	TAC	TCC	594
Trp	Thr	Pro	Gly	Ala	His	Gly	Glu	Thr	Phe	Leu	His	Thr	Asn	Tyr	Ser	
	155					160					165					
CTC	AAG	TAC	AAG	CTT	AGG	TGG	TAT	GGC	CAG	GAC	AAC	ACA	TGT	GAG	GAG	642
Leu	Lys	Tyr	Lys	Leu	Arg	Trp	Tyr	Gly	Gln	Asp	Asn	Thr	Cys	Glu	Glu	
170					175					180					185	
TAC	CAC	ACA	GTG	GGG	CCC	CAC	TCC	TGC	CAC	ATC	CCC	AAG	GAC	CTG	GCT	690
Tyr	His	Thr	Val	Gly	Pro	His	Ser	Cys	His	Ile	Pro	Lys	Asp	Leu	Ala	
			190						195					200		
CTC	TTT	ACG	CCC	TAT	GAG	ATC	TGG	GTG	GAG	GCC	ACC	AAC	CGC	CTG	GGC	738
Leu	Phe	Thr	Pro	Tyr	Glu	Ile	Trp	Val	Glu	Ala	Thr	Asn	Arg	Leu	Gly	
			205					210					215			
TCT	GCC	CGC	TCC	GAT	GTA	CTC	ACG	CTG	GAT	ATC	CTG	GAT	GTG	GTG	ACC	786
Ser	Ala	Arg	Ser	Asp	Val	Leu	Thr	Leu	Asp	Ile	Leu	Asp	Val	Val	Thr	
		220					225					230				
ACG	GAC	CCC	CCG	CCC	GAC	GTG	CAC	GTG	AGC	CGC	GTC	GGG	GGC	CTG	GAG	834
Thr	Asp	Pro	Pro	Pro	Asp	Val	His	Val	Ser	Arg	Val	Gly	Gly	Leu	Glu	
	235					240					245					
GAC	CAG	CTG	AGC	GTG	CGC	TGG	GTG	TCG	CCA	CCC	GCC	CTC	AAG	GAT	TTC	882
Asp	Gln	Leu	Ser	Val	Arg	Trp	Val	Ser	Pro	Pro	Ala	Leu	Lys	Asp	Phe	
250					255					260					265	
CTC	TTT	CAA	GCC	AAA	TAC	CAG	ATC	CGC	TAC	CGA	GTG	GAG	GAC	AGT	GTG	930
Leu	Phe	Gln	Ala	Lys	Tyr	Gln	Ile	Arg	Tyr	Arg	Val	Glu	Asp	Ser	Val	
			270					275						280		
GAC	TGG	AAG	GTG	GTG	GAC	GAT	GTG	AGC	AAC	CAG	ACC	TCC	TGC	CGC	CTG	978
Asp	Trp	Lys	Val	Val	Asp	Asp	Val	Ser	Asn	Gln	Thr	Ser	Cys	Arg	Leu	
			285					290					295			
GCC	GGC	CTG	AAA	CCC	GGC	ACC	GTG	TAC	TTC	GTG	CAA	GTG	CGC	TGC	AAC	1026
Ala	Gly	Leu	Lys	Pro	Gly	Thr	Val	Tyr	Phe	Val	Gln	Val	Arg	Cys	Asn	
		300					305					310				

70

CCC TTT GGC ATC TAT GGC TCC AAG AAA GCC GGG ATC TGG AGT GAG TGG Pro Phe Gly Ile Tyr Gly Ser Lys Lys Ala Gly Ile Trp Ser Glu Trp 315 320 325	1074
AGC CAC CCC ACA GCC GCC TCC ACT CCC CGC AGT GAG CGC CCG GGC CCG Ser His Pro Thr Ala Ala Ser Thr Pro Arg Ser Glu Arg Pro Gly Pro 330 335 340 345	1122
GGC GGC GGG GCG TGC GAA CCG CGG GGC GGA GAG CCG AGC TCG GGG CCG Gly Gly Gly Ala Cys Glu Pro Arg Gly Gly Glu Pro Ser Ser Gly Pro 350 355 360	1170
GTG CGG CGC GAG CTC AAG CAG TTC CTG GGC TGG CTC AAG AAG CAC GCG Val Arg Arg Glu Leu Lys Gln Phe Leu Gly Trp Leu Lys Lys His Ala 365 370 375	1218
TAC TGC TCC AAC CTC AGC TTC CGC CTC TAC GAC CAG TGG CGA GCC TGG Tyr Cys Ser Asn Leu Ser Phe Arg Leu Tyr Asp Gln Trp Arg Ala Trp 380 385 390	1266
ATG CAG AAG TCG CAC AAG ACC CGC AAC CAG CAC AGG ACG AGG GGA TCC Met Gln Lys Ser His Lys Thr Arg Asn Gln His Arg Thr Arg Gly Ser 395 400 405	1314
TGC CCT CGG GCA GAC GGG GCA CGG CGA GAG GTC CTG CCA GAT AAG CTG T Cys Pro Arg Ala Asp Gly Ala Arg Arg Glu Val Leu Pro Asp Lys Leu 410 415 420 425	1363
AGGGGCTCAG GCCACCCTCC CTGCCACGTG GAGACGCAGA GGCCGAACCC AAAGTGGGGC CACCTCTGTA CCCTCACTTC AGGGCACCTG AGCCACCCTC AGCAGGAGCT GGGGTGGCCC CTGAGCTCCA ACGGCCATAA CAGCTCTGAC TCCCACGTGA GGCCACCTTT GGGTGCACCC CAGTGGGTGT GTGTGTGTGT GTGAGGGTTG GTTGAGTTGC CTAGAACCCC TGCCAGGGCT GGGGGTGAGA AGGGGAGTCA TTAATCCCCA TTACCTAGGG CCCCTCCAAA AGAGTCCTTT TAAATAAATG AGCTATTTAG GTGCAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA TTCCCGGGGA	1423 1483 1543 1603 1663 1723 1783 1813

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 425 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

Met Pro Ala Gly Arg Arg Gly Pro Ala Ala Gln Ser Ala Arg Arg Pro
 1           5           10           15
Pro Pro Leu Leu Pro Leu Leu Leu Cys Val Leu Gly Ala Pro Arg
      20           25           30
Ala Gly Ser Gly Ala His Thr Ala Val Ile Ser Pro Gln Asp Pro Thr
      35           40           45
Leu Leu Ile Gly Ser Ser Leu Leu Ala Thr Cys Ser Val His Gly Asp
      50           55           60
Pro Pro Gly Ala Thr Ala Glu Gly Leu Tyr Trp Thr Leu Asn Gly Arg
65           70           75           80
Arg Leu Pro Pro Glu Leu Ser Arg Val Leu Asn Ala Ser Thr Leu Ala
      85           90           95
Leu Ala Leu Ala Asn Leu Asn Gly Ser Arg Gln Arg Ser Gly Asp Asn
      100          105          110
Leu Val Cys His Ala Arg Asp Gly Ser Ile Leu Ala Gly Ser Cys Leu
      115          120          125
Tyr Val Gly Leu Pro Pro Glu Lys Pro Val Asn Ile Ser Cys Trp Ser
      130          135          140
Lys Asn Met Lys Asp Leu Thr Cys Arg Trp Thr Pro Gly Ala His Gly
145          150          155          160
Glu Thr Phe Leu His Thr Asn Tyr Ser Leu Lys Tyr Lys Leu Arg Trp
      165          170          175
Tyr Gly Gln Asp Asn Thr Cys Glu Glu Tyr His Thr Val Gly Pro His
      180          185          190
Ser Cys His Ile Pro Lys Asp Leu Ala Leu Phe Thr Pro Tyr Glu Ile
      195          200          205
Trp Val Glu Ala Thr Asn Arg Leu Gly Ser Ala Arg Ser Asp Val Leu
      210          215          220
Thr Leu Asp Ile Leu Asp Val Val Thr Thr Asp Pro Pro Pro Asp Val
225          230          235          240
His Val Ser Arg Val Gly Gly Leu Glu Asp Gln Leu Ser Val Arg Trp
      245          250          255
Val Ser Pro Pro Ala Leu Lys Asp Phe Leu Phe Gln Ala Lys Tyr Gln
      260          265          270
Ile Arg Tyr Arg Val Glu Asp Ser Val Asp Trp Lys Val Val Asp Asp
      275          280          285
Val Ser Asn Gln Thr Ser Cys Arg Leu Ala Gly Leu Lys Pro Gly Thr
      290          295          300
Val Tyr Phe Val Gln Val Arg Cys Asn Pro Phe Gly Ile Tyr Gly Ser
305          310          315          320

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GAATTCGGCA	CGAGGAATTT	CGGCTGCTCA	GACTTGCTCC	GGCCTTCGCT	GTCCGCGCCC	60
AGTGACGTGC	GTGCGGACCC	AAACCCCAAT	CTGCACCCCG	CAGAGTCGCC	CCCGCCCCAT	120
ACCGGCGTTG	CAGTCAACGC	CCGTTGCGCG	CCACCCCC	ATG CCC GCC GGT GGC CCG		176
				Met Pro Ala Gly Gly Pro		
				1	5	
GGC CCC GCC GCC CAA TCC GCG CGG CGG CCG CCG CGG CGG CTC TCC TCG						224
Gly Pro Ala Ala Gln Ser Ala Arg Arg Pro Pro Arg Arg Leu Ser Ser						
	10		15		20	
CTG TGG TCG CCT CTG TTG CTC TGT GTT CTC GGG GTG CCT CAG GGC GGA						272
Leu Trp Ser Pro Leu Leu Leu Cys Val Leu Gly Val Pro Gln Gly Gly						
	25		30		35	

TCG GGA GCC CAC ACA GCT GTG ATC AGT CCC CAG GAC CCC ACT CTT CTC	320
Ser Gly Ala His Thr Ala Val Ile Ser Pro Gln Asp Pro Thr Leu Leu	
40 45 50	
ATC GGA TCC TCC CTT CAT GCT ACG TGC TCT ATA CAT GGA GAC ACA CCG	368
Ile Gly Ser Ser Leu His Ala Thr Cys Ser Ile His Gly Asp Thr Pro	
55 60 65 70	
GGG GCC ACT GCT GAG GGC CTC TAC TGG ACC CTC AAC GGC CGC CGC CTG	416
Gly Ala Thr Ala Glu Gly Leu Tyr Trp Thr Leu Asn Gly Arg Arg Leu	
75 80 85	
CCC TCA GAG CTG TCC CGT CTC CTC AAC ACC TCC ACC CTG GCC TTG GCC	464
Pro Ser Glu Leu Ser Arg Leu Leu Asn Thr Ser Thr Leu Ala Leu Ala	
90 95 100	
CTG GCT AAC CTT AAT GGG TCC AGG CAG CAG TCA GGG GAC AAT CTG GTG	512
Leu Ala Asn Leu Asn Gly Ser Arg Gln Gln Ser Gly Asp Asn Leu Val	
105 110 115	
TGT CAC GCC CGA GAT GGC AGC ATT CTG GCT GGT TCC TGC CTC TAT GTT	560
Cys His Ala Arg Asp Gly Ser Ile Leu Ala Gly Ser Cys Leu Tyr Val	
120 125 130	
GGT CTG CCC CCG GAG AAG CCC TTT AAC ATC AGC TGC TGG TCC CGG AAC	608
Gly Leu Pro Pro Glu Lys Pro Phe Asn Ile Ser Cys Trp Ser Arg Asn	
135 140 145 150	
ATG AAG GAC CTG ACA TGC CGT TGG ACA CCG GGT GCA CAT GGG GAG ACA	656
Met Lys Asp Leu Thr Cys Arg Trp Thr Pro Gly Ala His Gly Glu Thr	
155 160 165	
TTC CTA CAC ACC AAC TAC TCC CTC AAG TAC AAG CTG AGG TGG TAT GGT	704
Phe Leu His Thr Asn Tyr Ser Leu Lys Tyr Lys Leu Arg Trp Tyr Gly	
170 175 180	
CAG GAC AAC ACA TGT GAG GAA TAT CAC ACT GTG GGC CCT CAC TCG TGC	752
Gln Asp Asn Thr Cys Glu Glu Tyr His Thr Val Gly Pro His Ser Cys	
185 190 195	
CAT ATC CCC AAA GAC CTG GCC CTC TTC ACG CCC TAT GAG ATC TGG GTG	800
His Ile Pro Lys Asp Leu Ala Leu Phe Thr Pro Tyr Glu Ile Trp Val	
200 205 210	

GAA	GCC	ACC	AAT	CGC	CTG	GGT	TCA	GCG	AGA	TCT	GAC	GTG	CTC	ACA	CTG	848
Glu	Ala	Thr	Asn	Arg	Leu	Gly	Ser	Ala	Arg	Ser	Asp	Val	Leu	Thr	Leu	
215					220					225					230	
GAT	GTC	CTG	GAC	GTG	GTG	ACC	ACG	GAC	CCT	CCA	CCC	GAC	GTG	CAC	GTG	896
Asp	Val	Leu	Asp	Val	Val	Thr	Thr	Asp	Pro	Pro	Pro	Asp	Val	His	Val	
				235					240					245		
AGC	CGC	GTT	GGG	GGC	CTG	GAG	GAC	CAG	CTG	AGT	GTG	CGC	TGG	GTC	TCA	944
Ser	Arg	Val	Gly	Gly	Leu	Glu	Asp	Gln	Leu	Ser	Val	Arg	Trp	Val	Ser	
			250					255					260			
CCA	CCA	GCT	CTC	AAG	GAT	TTC	CTC	TTC	CAA	GCC	AAA	TAC	CAG	ATT	CGC	992
Pro	Pro	Ala	Leu	Lys	Asp	Phe	Leu	Phe	Gln	Ala	Lys	Tyr	Gln	Ile	Arg	
		265					270					275				
TAC	CGC	GTG	GAG	GAC	AGC	GTG	GAC	TGG	AAG	GTG	GTG	GAT	GAC	GTC	AGC	1040
Tyr	Arg	Val	Glu	Asp	Ser	Val	Asp	Trp	Lys	Val	Val	Asp	Asp	Val	Ser	
	280					285					290					
AAC	CAG	ACC	TCC	TGC	CGT	CTC	GCG	GGC	TTG	AAG	CCC	GGC	ACC	GTT	TAC	1088
Asn	Gln	Thr	Ser	Cys	Arg	Leu	Ala	Gly	Leu	Lys	Pro	Gly	Thr	Val	Tyr	
295					300					305					310	
TTC	GTC	CAA	GTT	CGT	TGT	AAC	CCA	TTC	GGG	ATC	TAT	GGG	TCG	AAA	AAG	1136
Phe	Val	Gln	Val	Arg	Cys	Asn	Pro	Phe	Gly	Ile	Tyr	Gly	Ser	Lys	Lys	
				315					320					325		
GCG	GGA	ATC	TGG	AGC	GAG	TGG	AGC	CAC	CCC	ACC	GCT	GCC	TCC	ACC	CCT	1184
Ala	Gly	Ile	Trp	Ser	Glu	Trp	Ser	His	Pro	Thr	Ala	Ala	Ser	Thr	Pro	
			330					335					340			
CGA	AGT	GAG	CGC	CCG	GGC	CCG	GGC	GGC	GGG	GTG	TGC	GAG	CCG	CGG	GGC	1232
Arg	Ser	Glu	Arg	Pro	Gly	Pro	Gly	Gly	Gly	Val	Cys	Glu	Pro	Arg	Gly	
		345					350					355				
GGC	GAG	CCT	AGC	TCG	GGC	CCG	GTG	CGG	CGC	GAG	CTC	AAG	CAG	TTC	CTC	1280
Gly	Glu	Pro	Ser	Ser	Gly	Pro	Val	Arg	Arg	Glu	Leu	Lys	Gln	Phe	Leu	
	360					365					370					
GGC	TGG	CTC	AAG	AAG	CAC	GCG	TAC	TGC	TCG	AAC	CTT	AGC	TTC	CGC	CTG	1328
Gly	Trp	Leu	Lys	Lys	His	Ala	Tyr	Cys	Ser	Asn	Leu	Ser	Phe	Arg	Leu	
375					380					385					390	

75

TAC GAC CAG TGG CGT GCT TGG ATG CAG AAG TCA CAC AAG ACC CGA AAC 1376
 Tyr Asp Gln Trp Arg Ala Trp Met Gln Lys Ser His Lys Thr Arg Asn
 395 400 405

CAG GAC GAG GGG ATC CTG CCC TCG GGC AGA CGG GGT GCG GCG AGA GGT 1424
 Gln Asp Glu Gly Ile Leu Pro Ser Gly Arg Arg Gly Ala Ala Arg Gly
 410 415 420

CCT GCC GGC TAAACTCTGA GGATAGGCCA TCCTCCTGCT GGATGCAGAC CTGGAGGCT 1482
 Pro Ala Gly
 425

CACCTGAACT GGAGACCATC TGTACTGTCA CTTTGGGGCA ATGAAGAAAC AAACCAGGGG 1542
 CTGGGGCACA ATGAGCTCCC ACAACCACAG CTTTGGCCAC ATGATGGTCA ACTTTGGATG 1602
 TACCCCAATA TGGGTAGGGT TGGAGTAATG ACAAGGGTTA TGCAGGACCC TCCAAGAGTC 1662
 TCTTTGAATA AATAAGAAAA GAGTTGTTCA GGAAAAA AAAA AATAGCGGCC 1722
 GC 1724

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 425 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Pro Ala Gly Gly Pro Gly Pro Ala Ala Gln Ser Ala Arg Arg Pro
 1 5 10 15
 Pro Arg Arg Leu Ser Ser Leu Trp Ser Pro Leu Leu Leu Cys Val Leu
 20 25 30
 Gly Val Pro Gln Gly Gly Ser Gly Ala His Thr Ala Val Ile Ser Pro
 35 40 45
 Gln Asp Pro Thr Leu Leu Ile Gly Ser Ser Leu His Ala Thr Cys Ser
 50 55 60
 Ile His Gly Asp Thr Pro Gly Ala Thr Ala Glu Gly Leu Tyr Trp Thr
 65 70 75 80
 Leu Asn Gly Arg Arg Leu Pro Ser Glu Leu Ser Arg Leu Leu Asn Thr
 85 90 95
 Ser Thr Leu Ala Leu Ala Leu Ala Asn Leu Asn Gly Ser Arg Gln Gln
 100 105 110

Ser	Gly	Asp	Asn	Leu	Val	Cys	His	Ala	Arg	Asp	Gly	Ser	Ile	Leu	Ala
		115					120					125			
Gly	Ser	Cys	Leu	Tyr	Val	Gly	Leu	Pro	Pro	Glu	Lys	Pro	Phe	Asn	Ile
		130				135					140				
Ser	Cys	Trp	Ser	Arg	Asn	Met	Lys	Asp	Leu	Thr	Cys	Arg	Trp	Thr	Pro
145					150					155					160
Gly	Ala	His	Gly	Glu	Thr	Phe	Leu	His	Thr	Asn	Tyr	Ser	Leu	Lys	Tyr
			165						170					175	
Lys	Leu	Arg	Trp	Tyr	Gly	Gln	Asp	Asn	Thr	Cys	Glu	Glu	Tyr	His	Thr
		180						185					190		
Val	Gly	Pro	His	Ser	Cys	His	Ile	Pro	Lys	Asp	Leu	Ala	Leu	Phe	Thr
		195					200					205			
Pro	Tyr	Glu	Ile	Trp	Val	Glu	Ala	Thr	Asn	Arg	Leu	Gly	Ser	Ala	Arg
		210				215					220				
Ser	Asp	Val	Leu	Thr	Leu	Asp	Val	Leu	Asp	Val	Val	Thr	Thr	Asp	Pro
225					230					235					240
Pro	Pro	Asp	Val	His	Val	Ser	Arg	Val	Gly	Gly	Leu	Glu	Asp	Gln	Leu
			245						250					255	
Ser	Val	Arg	Trp	Val	Ser	Pro	Pro	Ala	Leu	Lys	Asp	Phe	Leu	Phe	Gln
		260						265					270		
Ala	Lys	Tyr	Gln	Ile	Arg	Tyr	Arg	Val	Glu	Asp	Ser	Val	Asp	Trp	Lys
		275					280					285			
Val	Val	Asp	Asp	Val	Ser	Asn	Gln	Thr	Ser	Cys	Arg	Leu	Ala	Gly	Leu
		290				295					300				
Lys	Pro	Gly	Thr	Val	Tyr	Phe	Val	Gln	Val	Arg	Cys	Asn	Pro	Phe	Gly
305					310					315					320
Ile	Tyr	Gly	Ser	Lys	Lys	Ala	Gly	Ile	Trp	Ser	Glu	Trp	Ser	His	Pro
			325						330					335	
Thr	Ala	Ala	Ser	Thr	Pro	Arg	Ser	Glu	Arg	Pro	Gly	Pro	Gly	Gly	Gly
			340					345					350		
Val	Cys	Glu	Pro	Arg	Gly	Gly	Glu	Pro	Ser	Ser	Gly	Pro	Val	Arg	Arg
		355					360				365				
Glu	Leu	Lys	Gln	Phe	Leu	Gly	Trp	Leu	Lys	Lys	His	Ala	Tyr	Cys	Ser
		370				375					380				
Asn	Leu	Ser	Phe	Arg	Leu	Tyr	Asp	Gln	Trp	Arg	Ala	Trp	Met	Gln	Lys
385					390					395					400
Ser	His	Lys	Thr	Arg	Asn	Gln	Asp	Glu	Gly	Ile	Leu	Pro	Ser	Gly	Arg
			405						410					415	
Arg	Gly	Ala	Ala	Arg	Gly	Pro	Ala	Gly							
			420					425							

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 259 base pairs

77

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

AGGATTTCTT	CTTTCAAGCC	AAATACCAGA	TCCGCTANCG	AGTGGAGGAN	AGTGTGGANT	60
GGAAGGTGGT	GGANGATGTG	AGCAACCAGA	CCTTCTGCCG	CTGGNCGGCC	TGAAACCCGG	120
CANCGTGAC	TTCGTGCAAG	TGCGCTGCAA	NCCCTTTGGC	ATCTATGGCT	NCAAGAAAGC	180
CGGGATCTNG	AGTGAGTGGA	GCCANCCAC	AGCCGGCTTC	ANTTCCCGCA	GTGAGCGNCN	240
GGGCCCGGGN	GGNGGGAAG					259

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CGCGAGCTCA	AGCAGTTCCT	G	21
------------	------------	---	----

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 210 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GNACACGCCG	NTATAGCTNG	CCCCTGCTGC	TGCTGCTCTG	CGTCCTCGGG	GCNCGCGAGC	60
GGATTCAGGA	GCCCACACAG	CTGTGATCAG	TCCCCAGGAT	CCCACGCTTC	TCATCGGCTC	120
CTCCCTGCTG	GCCACCTGCT	CAGTGACCGG	AGACCCACCA	GGAGCCACCG	CCGAGGGCCT	180
CTACTGGACC	CTCAACGGGC	GCCGCTGCCC				210

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CCACCTAAGC TTGTACTTGA GG

22

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AGCCACACA GCTGTGATCA G

21

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 44 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GACAGAGCAC AGAATTCAC TGTGAGCTCT TTTTTTTTTT TTTT

44

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TATGGCCAGG ACAACACA

18

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

ATAGGGCGTA AAGAGAGC

18

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TCACATCGTC CACCACCTTC CAGTCCA

27

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 49 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

80

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GAGCACAGAA TTCACTACTC GAGGCGGCCG CTTTTTTTTT TTTTTTTT

49

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 388 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Gly	Ser	Gly	Ala	His	Thr	Ala	Val	Ile	Ser	Pro	Gln	Asp	Pro	Thr	Leu
1				5				10						15	
Leu	Ile	Gly	Ser	Ser	Leu	Leu	Ala	Thr	Cys	Ser	Val	His	Gly	Asp	Pro
			20					25					30		
Pro	Gly	Ala	Thr	Ala	Glu	Gly	Leu	Tyr	Trp	Thr	Leu	Xaa	Gly	Arg	Arg
		35				40					45				
Leu	Pro	Pro	Glu	Leu	Ser	Arg	Val	Leu	Asn	Ala	Ser	Thr	Leu	Ala	Leu
	50				55				60						
Ala	Leu	Ala	Asn	Leu	Asn	Gly	Ser	Arg	Gln	Arg	Ser	Gly	Asp	Asn	Leu
65				70				75						80	
Val	Cys	His	Ala	Arg	Asp	Gly	Ser	Ile	Leu	Ala	Gly	Ser	Cys	Leu	Tyr
			85					90					95		
Val	Gly	Leu	Pro	Pro	Glu	Lys	Pro	Val	Asn	Ile	Ser	Cys	Trp	Ser	Lys
		100						105				110			
Asn	Met	Lys	Asp	Leu	Thr	Cys	Arg	Trp	Thr	Pro	Gly	Ala	His	Gly	Glu
	115						120					125			
Thr	Phe	Leu	His	Thr	Asn	Tyr	Ser	Leu	Lys	Tyr	Lys	Leu	Arg	Trp	Tyr
	130					135				140					
Gly	Gln	Asp	Asn	Thr	Cys	Glu	Glu	Tyr	His	Thr	Val	Gly	Pro	His	Ser
145				150					155					160	
Cys	His	Ile	Pro	Lys	Asp	Leu	Ala	Leu	Phe	Thr	Pro	Tyr	Glu	Ile	Trp
			165					170					175		
Val	Glu	Ala	Thr	Asn	Arg	Leu	Gly	Ser	Ala	Arg	Ser	Asp	Val	Leu	Thr
		180						185					190		

Leu Asp Ile Leu Asp Val Val Thr Thr Asp Pro Pro Pro Glu Val His
 195 200 205
 Val Ser Arg Val Gly Gly Leu Glu Asp Gln Leu Ser Val Arg Trp Val
 210 215 220
 Ser Pro Pro Ala Leu Lys Asp Phe Leu Phe Gln Ala Lys Tyr Gln Ile
 225 230 235 240
 Arg Tyr Arg Val Glu Asp Ser Val Asp Trp Lys Val Val Asp Asp Val
 245 250 255
 Ser Asn Gln Thr Ser Cys Arg Leu Ala Gly Leu Lys Pro Gly Thr Val
 260 265 270
 Tyr Phe Val Gln Val Arg Cys Asn Pro Phe Gly Ile Tyr Gly Ser Lys
 275 280 285
 Lys Ala Gly Ile Trp Ser Glu Trp Ser His Pro Thr Ala Ala Ser Thr
 290 295 300
 Pro Arg Ser Glu Arg Pro Gly Pro Gly Gly Gly Ala Cys Glu Pro Arg
 305 310 315 320
 Gly Gly Glu Pro Ser Ser Gly Pro Val Arg Arg Glu Leu Lys Gln Phe
 325 330 335
 Leu Gly Trp Leu Lys Lys His Ala Tyr Cys Ser Asn Leu Ser Phe Arg
 340 345 350
 Leu Tyr Asp Gln Trp Arg Ala Trp Met Gln Lys Ser His Lys Thr Arg
 355 360 365
 Asn Gln Asp Glu Gly Ile Leu Pro Ser Gly Arg Arg Gly Thr Ala Arg
 370 375 380
 Gly Pro Ala Arg
 385

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 392 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Gly Ser Gly Ala His Thr Ala Val Ile Ser Pro Gln Asp Pro Thr Leu
 1 5 10 15
 Leu Ile Gly Ser Ser Leu Leu Ala Thr Cys Ser Val His Gly Asp Pro
 20 25 30
 Pro Gly Ala Thr Ala Glu Gly Leu Tyr Trp Thr Leu Asn Gly Arg Arg
 35 40 45

Leu Pro Pro Glu Leu Ser Arg Val Leu Asn Ala Ser Thr Leu Ala Leu
 50 55 60
 Ala Leu Ala Asn Leu Asn Gly Ser Arg Gln Arg Ser Gly Asp Asn Leu
 65 70 75 80
 Val Cys His Ala Arg Asp Gly Ser Ile Leu Ala Gly Ser Cys Leu Tyr
 85 90 95
 Val Gly Leu Pro Pro Glu Lys Pro Val Asn Ile Ser Cys Trp Ser Lys
 100 105 110
 Asn Met Lys Asp Leu Thr Cys Arg Trp Thr Pro Gly Ala His Gly Glu
 115 120 125
 Thr Phe Leu His Thr Asn Tyr Ser Leu Lys Tyr Lys Leu Arg Trp Tyr
 130 135 140
 Gly Gln Asp Asn Thr Cys Glu Glu Tyr His Thr Val Gly Pro His Ser
 145 150 155 160
 Cys His Ile Pro Lys Asp Leu Ala Leu Phe Thr Pro Tyr Glu Ile Trp
 165 170 175
 Val Glu Ala Thr Asn Arg Leu Gly Ser Ala Arg Ser Asp Val Leu Thr
 180 185 190
 Leu Asp Ile Leu Asp Val Val Thr Thr Asp Pro Pro Pro Asp Val His
 195 200 205
 Val Ser Arg Val Gly Gly Leu Glu Asp Gln Leu Ser Val Arg Trp Val
 210 215 220
 Ser Pro Pro Ala Leu Lys Asp Phe Leu Phe Gln Ala Lys Tyr Gln Ile
 225 230 235 240
 Arg Tyr Arg Val Glu Asp Ser Val Asp Trp Lys Val Val Asp Asp Val
 245 250 255
 Ser Asn Gln Thr Ser Cys Arg Leu Ala Gly Leu Lys Pro Gly Thr Val
 260 265 270
 Tyr Phe Val Gln Val Arg Cys Asn Pro Phe Gly Ile Tyr Gly Ser Lys
 275 280 285
 Lys Ala Gly Ile Trp Ser Glu Trp Ser His Pro Thr Ala Ala Ser Thr
 290 295 300
 Pro Arg Ser Glu Arg Pro Gly Pro Gly Gly Gly Ala Cys Glu Pro Arg
 305 310 315 320
 Gly Gly Glu Pro Ser Ser Gly Pro Val Arg Arg Glu Leu Lys Gln Phe
 325 330 335
 Leu Gly Trp Leu Lys Lys His Ala Tyr Cys Ser Asn Leu Ser Phe Arg
 340 345 350
 Leu Tyr Asp Gln Trp Arg Ala Trp Met Gln Lys Ser His Lys Thr Arg
 355 360 365
 Asn Gln His Arg Thr Arg Gly Ser Cys Pro Arg Ala Asp Gly Ala Arg
 370 375 380
 Arg Glu Val Leu Pro Asp Lys Leu
 385 390

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 385 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```

Ala His Thr Ala Val Ile Ser Pro Gln Asp Pro Thr Leu Leu Ile Gly
 1           5           10           15
Ser Ser Leu His Ala Thr Cys Ser Ile His Gly Asp Thr Pro Gly Ala
 20           25           30
Thr Ala Glu Gly Leu Tyr Trp Thr Leu Asn Gly Arg Arg Leu Pro Ser
 35           40           45
Glu Leu Ser Arg Leu Leu Asn Thr Ser Thr Leu Ala Leu Ala Leu Ala
 50           55           60
Asn Leu Asn Gly Ser Arg Gln Gln Ser Gly Asp Asn Leu Val Cys His
 65           70           75           80
Ala Arg Asp Gly Ser Ile Leu Ala Gly Ser Cys Leu Tyr Val Gly Leu
 85           90           95
Pro Pro Glu Lys Pro Phe Asn Ile Ser Cys Trp Ser Arg Asn Met Lys
100          105          110
Asp Leu Thr Cys Arg Trp Thr Pro Gly Ala His Gly Glu Thr Phe Leu
115          120          125
His Thr Asn Tyr Ser Leu Lys Tyr Lys Leu Arg Trp Tyr Gly Gln Asp
130          135          140
Asn Thr Cys Glu Glu Tyr His Thr Val Gly Pro His Ser Cys His Ile
145          150          155          160
Pro Lys Asp Leu Ala Leu Phe Thr Pro Tyr Glu Ile Trp Val Glu Ala
165          170          175
Thr Asn Arg Leu Gly Ser Ala Arg Ser Asp Val Leu Thr Leu Asp Val
180          185          190
Leu Asp Val Val Thr Thr Asp Pro Pro Pro Asp Val His Val Ser Arg
195          200          205
Val Gly Gly Leu Glu Asp Gln Leu Ser Val Arg Trp Val Ser Pro Pro
210          215          220
Ala Leu Lys Asp Phe Leu Phe Gln Ala Lys Tyr Gln Ile Arg Tyr Arg
225          230          235          240
Val Glu Asp Ser Val Asp Trp Lys Val Val Asp Asp Val Ser Asn Gln
245          250          255

```

Thr Ser Cys Arg Leu Ala Gly Leu Lys Pro Gly Thr Val Tyr Phe Val
 260 265 270
 Gln Val Arg Cys Asn Pro Phe Gly Ile Tyr Gly Ser Lys Lys Ala Gly
 275 280 285
 Ile Trp Ser Glu Trp Ser His Pro Thr Ala Ala Ser Thr Pro Arg Ser
 290 295 300
 Glu Arg Pro Gly Pro Gly Gly Gly Val Cys Glu Pro Arg Gly Gly Glu
 305 310 315 320
 Pro Ser Ser Gly Pro Val Arg Arg Glu Leu Lys Gln Phe Leu Gly Trp
 325 330 335
 Leu Lys Lys His Ala Tyr Cys Ser Asn Leu Ser Phe Arg Leu Tyr Asp
 340 345 350
 Gln Trp Arg Ala Trp Met Gln Lys Ser His Lys Thr Arg Asn Gln Asp
 355 360 365
 Glu Gly Ile Leu Pro Ser Gly Arg Arg Gly Ala Ala Arg Gly Pro Ala
 370 375 380
 Gly
 385

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 385 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Ala His Thr Ala Val Ile Ser Pro Gln Asp Pro Thr Leu Leu Ile Gly
 1 5 10 15
 Ser Ser Leu Leu Ala Thr Cys Ser Val His Gly Asp Pro Pro Gly Ala
 20 25 30
 Thr Ala Glu Gly Leu Tyr Trp Thr Leu Xaa Gly Arg Arg Leu Pro Pro
 35 40 45
 Glu Leu Ser Arg Val Leu Asn Ala Ser Thr Leu Ala Leu Ala Leu Ala
 50 55 60
 Asn Leu Asn Gly Ser Arg Gln Arg Ser Gly Asp Asn Leu Val Cys His
 65 70 75 80
 Ala Arg Asp Gly Ser Ile Leu Ala Gly Ser Cys Leu Tyr Val Gly Leu
 85 90 95
 Pro Pro Glu Lys Pro Val Asn Ile Ser Cys Trp Ser Lys Asn Met Lys
 100 105 110

Asp Leu Thr Cys Arg Trp Thr Pro Gly Ala His Gly Glu Thr Phe Leu
 115 120 125
 His Thr Asn Tyr Ser Leu Lys Tyr Lys Leu Arg Trp Tyr Gly Gln Asp
 130 135 140
 Asn Thr Cys Glu Glu Tyr His Thr Val Gly Pro His Ser Cys His Ile
 145 150 155 160
 Pro Lys Asp Leu Ala Leu Phe Thr Pro Tyr Glu Ile Trp Val Glu Ala
 165 170 175
 Thr Asn Arg Leu Gly Ser Ala Arg Ser Asp Val Leu Thr Leu Asp Ile
 180 185 190
 Leu Asp Val Val Thr Thr Asp Pro Pro Pro Glu Val His Val Ser Arg
 195 200 205
 Val Gly Gly Leu Glu Asp Gln Leu Ser Val Arg Trp Val Ser Pro Pro
 210 215 220
 Ala Leu Lys Asp Phe Leu Phe Gln Ala Lys Tyr Gln Ile Arg Tyr Arg
 225 230 235 240
 Val Glu Asp Ser Val Asp Trp Lys Val Val Asp Asp Val Ser Asn Gln
 245 250 255
 Thr Ser Cys Arg Leu Ala Gly Leu Lys Pro Gly Thr Val Tyr Phe Val
 260 265 270
 Gln Val Arg Cys Asn Pro Phe Gly Ile Tyr Gly Ser Lys Lys Ala Gly
 275 280 285
 Ile Trp Ser Glu Trp Ser His Pro Thr Ala Ala Ser Thr Pro Arg Ser
 290 295 300
 Glu Arg Pro Gly Pro Gly Gly Gly Ala Cys Glu Pro Arg Gly Gly Glu
 305 310 315 320
 Pro Ser Ser Gly Pro Val Arg Arg Glu Leu Lys Gln Phe Leu Gly Trp
 325 330 335
 Leu Lys Lys His Ala Tyr Cys Ser Asn Leu Ser Phe Arg Leu Tyr Asp
 340 345 350
 Gln Trp Arg Ala Trp Met Gln Lys Ser His Lys Thr Arg Asn Gln Asp
 355 360 365
 Glu Gly Ile Leu Pro Ser Gly Arg Arg Gly Thr Ala Arg Gly Pro Ala
 370 375 380
 Arg
 385

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 303 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```

Ala His Thr Ala Val Ile Ser Pro Gln Asp Pro Thr Leu Leu Ile Gly
 1           5           10           15
Ser Ser Leu Leu Ala Thr Cys Ser Val His Gly Asp Pro Pro Gly Ala
      20           25           30
Thr Ala Glu Gly Leu Tyr Trp Thr Leu Asn Gly Arg Arg Leu Pro Pro
      35           40           45
Glu Leu Ser Arg Val Leu Asn Ala Ser Thr Leu Ala Leu Ala Leu Ala
 50           55           60
Asn Leu Asn Gly Ser Arg Gln Arg Ser Gly Asp Asn Leu Val Cys His
65           70           75           80
Ala Arg Asp Gly Ser Ile Leu Ala Gly Ser Cys Leu Tyr Val Gly Leu
      85           90           95
Pro Pro Glu Lys Pro Val Asn Ile Ser Cys Trp Ser Lys Asn Met Lys
      100          105          110
Asp Leu Thr Cys Arg Trp Thr Pro Gly Ala His Gly Glu Thr Phe Leu
      115          120          125
His Thr Asn Tyr Ser Leu Lys Tyr Lys Leu Arg Trp Tyr Gly Gln Asp
130          135          140
Asn Thr Cys Glu Glu Tyr His Thr Val Gly Pro His Ser Cys His Ile
145          150          155          160
Pro Lys Asp Leu Ala Leu Phe Thr Pro Tyr Glu Ile Trp Val Glu Ala
      165          170          175
Thr Asn Arg Leu Gly Ser Ala Arg Ser Asp Val Leu Thr Leu Asp Ile
      180          185          190
Leu Asp Val Val Thr Thr Asp Pro Pro Pro Glu Val His Val Ser Arg
      195          200          205
Val Gly Gly Leu Glu Asp Gln Leu Ser Val Arg Trp Val Ser Pro Pro
      210          215          220
Ala Leu Lys Asp Phe Leu Phe Gln Ala Lys Tyr Gln Ile Arg Tyr Arg
225          230          235          240
Val Glu Asp Ser Val Asp Trp Lys Val Val Asp Asp Val Ser Asn Gln
      245          250          255
Thr Ser Cys Arg Leu Ala Gly Leu Lys Pro Gly Thr Val Tyr Phe Val
      260          265          270
Gln Val Arg Cys Asn Pro Phe Gly Ile Tyr Gly Ser Lys Lys Ala Gly
      275          280          285
Ile Trp Ser Glu Trp Ser His Pro Thr Ala Ala Ser Thr Pro Arg
      290          295          300

```

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 389 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```

Ala His Thr Ala Val Ile Ser Pro Gln Asp Pro Thr Leu Leu Ile Gly
 1           5           10           15
Ser Ser Leu Leu Ala Thr Cys Ser Val His Gly Asp Pro Pro Gly Ala
          20           25           30
Thr Ala Glu Gly Leu Tyr Trp Thr Leu Asn Gly Arg Arg Leu Pro Pro
          35           40           45
Glu Leu Ser Arg Val Leu Asn Ala Ser Thr Leu Ala Leu Ala Leu Ala
          50           55           60
Asn Leu Asn Gly Ser Arg Gln Arg Ser Gly Asp Asn Leu Val Cys His
65           70           75           80
Ala Arg Asp Gly Ser Ile Leu Ala Gly Ser Cys Leu Tyr Val Gly Leu
          85           90           95
Pro Pro Glu Lys Pro Val Asn Ile Ser Cys Trp Ser Lys Asn Met Lys
          100          105          110
Asp Leu Thr Cys Arg Trp Thr Pro Gly Ala His Gly Glu Thr Phe Leu
          115          120          125
His Thr Asn Tyr Ser Leu Lys Tyr Lys Leu Arg Trp Tyr Gly Gln Asp
          130          135          140
Asn Thr Cys Glu Glu Tyr His Thr Val Gly Pro His Ser Cys His Ile
145          150          155          160
Pro Lys Asp Leu Ala Leu Phe Thr Pro Tyr Glu Ile Trp Val Glu Ala
          165          170          175
Thr Asn Arg Leu Gly Ser Ala Arg Ser Asp Val Leu Thr Leu Asp Ile
          180          185          190
Leu Asp Val Val Thr Thr Asp Pro Pro Pro Asp Val His Val Ser Arg
          195          200          205
Val Gly Gly Leu Glu Asp Gln Leu Ser Val Arg Trp Val Ser Pro Pro
          210          215          220
Ala Leu Lys Asp Phe Leu Phe Gln Ala Lys Tyr Gln Ile Arg Tyr Arg
225          230          235          240
Val Glu Asp Ser Val Asp Trp Lys Val Val Asp Asp Val Ser Asn Gln
          245          250          255
Thr Ser Cys Arg Leu Ala Gly Leu Lys Pro Gly Thr Val Tyr Phe Val
          260          265          270

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[illegible]

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 303 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Ala 1	His	Thr	Ala	Val 5	Ile	Ser	Pro	Gln	Asp 10	Pro	Thr	Leu	Leu	Ile 15	Gly
Ser	Ser	Leu	Leu	Ala 20	Thr	Cys	Ser	Val 25	His	Gly	Asp	Pro	Pro	Gly 30	Ala
Thr	Ala	Glu	Gly	Leu	Tyr	Trp	Thr 40	Leu	Asn	Gly	Arg	Arg	Leu	Pro	Pro
Glu	Leu	Ser	Arg	Val 50	Leu	Asn 55	Ala	Ser	Thr	Leu	Ala 60	Leu	Ala	Leu	Ala
Asn 65	Leu	Asn	Gly	Ser 70	Arg	Gln	Arg	Ser	Gly	Asp 75	Asn	Leu	Val	Cys 80	His
Ala	Arg	Asp	Gly	Ser 85	Ile	Leu	Ala	Gly	Ser 90	Cys	Leu	Tyr	Val	Gly 95	Leu
Pro	Pro	Glu	Lys	Pro 100	Val	Asn	Ile	Ser 105	Cys	Trp	Ser	Lys	Asn	Met 110	Lys
Asp	Leu	Thr	Cys	Arg 115	Trp	Thr	Pro 120	Gly	Ala	His	Gly	Glu	Thr	Phe	Leu

```

His Thr Asn Tyr Ser Leu Lys Tyr Lys Leu Arg Trp Tyr Gly Gln Asp
 130                      135                      140
Asn Thr Cys Glu Glu Tyr His Thr Val Gly Pro His Ser Cys His Ile
145                      150                      155                      160
Pro Lys Asp Leu Ala Leu Phe Thr Pro Tyr Glu Ile Trp Val Glu Ala
                      165                      170                      175
Thr Asn Arg Leu Gly Ser Ala Arg Ser Asp Val Leu Thr Leu Asp Ile
                      180                      185                      190
Leu Asp Val Val Thr Thr Asp Pro Pro Pro Asp Val His Val Ser Arg
                      195                      200                      205
Val Gly Gly Leu Glu Asp Gln Leu Ser Val Arg Trp Val Ser Pro Pro
210                      215                      220
Ala Leu Lys Asp Phe Leu Phe Gln Ala Lys Tyr Gln Ile Arg Tyr Arg
225                      230                      235                      240
Val Glu Asp Ser Val Asp Trp Lys Val Val Asp Asp Val Ser Asn Gln
                      245                      250                      255
Thr Ser Cys Arg Leu Ala Gly Leu Lys Pro Gly Thr Val Tyr Phe Val
                      260                      265                      270
Gln Val Arg Cys Asn Pro Phe Gly Ile Tyr Gly Ser Lys Lys Ala Gly
275                      280                      285
Ile Trp Ser Glu Trp Ser His Pro Thr Ala Ala Ser Thr Pro Arg
290                      295                      300

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(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 389 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```

Ala His Thr Ala Val Ile Ser Pro Gln Asp Pro Thr Leu Leu Ile Gly
 1                      5                      10                      15
Ser Ser Leu Leu Ala Thr Cys Ser Val His Gly Asp Pro Pro Gly Ala
                      20                      25                      30
Thr Ala Glu Gly Leu Tyr Trp Thr Leu Asn Gly Arg Arg Leu Pro Pro
                      35                      40                      45
Glu Leu Ser Arg Val Leu Asn Ala Ser Thr Leu Ala Leu Ala Leu Ala
50                      55                      60
Asn Leu Asn Gly Ser Arg Gln Arg Ser Gly Asp Asn Leu Val Cys His
65                      70                      75                      80

```

Ala Arg Asp Gly Ser Ile Leu Ala Gly Ser Cys Leu Tyr Val Gly Leu
 85 90 95
 Pro Pro Glu Lys Pro Val Asn Ile Ser Cys Trp Ser Lys Asn Met Lys
 100 105 110
 Asp Leu Thr Cys Arg Trp Thr Pro Gly Ser His Gly Glu Thr Phe Leu
 115 120 125
 His Thr Asn Tyr Ser Leu Lys Tyr Lys Leu Arg Trp Tyr Gly Gln Asp
 130 135 140
 Asn Thr Cys Glu Glu Tyr His Thr Val Gly Pro His Ser Cys His Ile
 145 150 155 160
 Pro Lys Asp Leu Ala Leu Phe Thr Pro Tyr Glu Ile Trp Val Glu Ala
 165 170 175
 Thr Asn Arg Leu Gly Ser Ala Arg Ser Asp Val Leu Thr Leu Asp Ile
 180 185 190
 Leu Asp Val Val Thr Thr Asp Pro Pro Pro Asp Val His Val Ser Arg
 195 200 205
 Val Gly Gly Leu Glu Asp Gln Leu Ser Val Arg Trp Val Ser Pro Pro
 210 215 220
 Ala Leu Lys Asp Phe Leu Phe Gln Ala Lys Tyr Gln Ile Arg Tyr Arg
 225 230 235 240
 Val Glu Asp Ser Val Asp Trp Lys Val Val Asp Asp Val Ser Asn Gln
 245 250 255
 Thr Ser Cys Arg Leu Ala Gly Leu Lys Pro Gly Thr Val Tyr Phe Val
 260 265 270
 Gln Val Arg Cys Asn Pro Phe Gly Ile Tyr Gly Ser Lys Lys Ala Gly
 275 280 285
 Ile Trp Ser Glu Trp Ser His Pro Thr Ala Ala Ser Thr Pro Arg Ser
 290 295 300
 Glu Arg Pro Gly Pro Gly Gly Gly Ala Cys Glu Pro Arg Gly Gly Glu
 305 310 315 320
 Pro Ser Ser Gly Pro Val Arg Arg Glu Leu Lys Gln Phe Leu Gly Trp
 325 330 335
 Leu Lys Lys His Ala Tyr Cys Ser Asn Leu Ser Phe Arg Leu Tyr Asp
 340 345 350
 Gln Trp Arg Ala Trp Met Gln Lys Ser His Lys Thr Arg Asn Gln His
 355 360 365
 Arg Thr Arg Gly Ser Cys Pro Arg Ala Asp Gly Ala Arg Arg Glu Val
 370 375 380
 Leu Pro Asp Lys Leu
 385

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 389 amino acids

(B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Ala	His	Thr	Ala	Val	Ile	Ser	Pro	Gln	Asp	Pro	Thr	Leu	Leu	Ile	Gly
1				5					10					15	
Ser	Ser	Leu	Leu	Ala	Thr	Cys	Ser	Val	His	Gly	Asp	Pro	Pro	Gly	Ala
		20						25					30		
Thr	Ala	Glu	Gly	Leu	Tyr	Trp	Thr	Leu	Asn	Gly	Arg	Arg	Leu	Pro	Pro
		35					40					45			
Glu	Leu	Ser	Arg	Val	Leu	Asn	Ala	Ser	Thr	Leu	Ala	Leu	Ala	Leu	Ala
	50					55				60					
Asn	Leu	Asn	Gly	Ser	Arg	Gln	Arg	Ser	Gly	Asp	Asn	Leu	Val	Cys	His
65					70				75					80	
Ala	Arg	Asp	Gly	Ser	Ile	Leu	Ala	Gly	Ser	Cys	Leu	Tyr	Val	Gly	Leu
				85					90					95	
Pro	Pro	Glu	Lys	Pro	Val	Asn	Ile	Ser	Cys	Trp	Ser	Lys	Asn	Met	Lys
		100						105					110		
Asp	Leu	Thr	Cys	Arg	Trp	Thr	Pro	Gly	Ala	His	Gly	Glu	Thr	Phe	Leu
		115					120					125			
His	Thr	Asn	Tyr	Ser	Leu	Lys	Tyr	Lys	Leu	Arg	Trp	Tyr	Gly	Gln	Asp
	130					135						140			
Asn	Thr	Cys	Glu	Asp	Tyr	His	Thr	Val	Gly	Pro	His	Ser	Cys	His	Ile
145					150				155					160	
Pro	Lys	Asp	Leu	Ala	Leu	Phe	Thr	Pro	Tyr	Glu	Ile	Trp	Val	Glu	Ala
				165					170					175	
Thr	Asn	Arg	Leu	Gly	Ser	Ala	Arg	Ser	Asp	Val	Leu	Thr	Leu	Asp	Ile
		180						185					190		
Leu	Asp	Val	Val	Thr	Thr	Asp	Pro	Pro	Pro	Asp	Val	His	Val	Ser	Arg
	195						200					205			
Val	Gly	Gly	Leu	Glu	Asp	Gln	Leu	Ser	Val	Arg	Trp	Val	Ser	Pro	Pro
	210					215					220				
Ala	Leu	Lys	Asp	Phe	Leu	Phe	Gln	Ala	Lys	Tyr	Gln	Ile	Arg	Tyr	Arg
225					230					235				240	
Val	Glu	Asp	Ser	Val	Asp	Trp	Lys	Val	Val	Asp	Asp	Val	Ser	Asn	Gln
				245					250					255	
Thr	Ser	Cys	Arg	Leu	Ala	Gly	Leu	Lys	Pro	Gly	Thr	Val	Tyr	Phe	Val
		260						265					270		
Gln	Val	Arg	Cys	Asn	Pro	Phe	Gly	Ile	Tyr	Gly	Ser	Lys	Lys	Ala	Gly
	275						280						285		

Ile Trp Ser Glu Trp Ser His Pro Thr Ala Ala Ser Thr Pro Arg Ser
 290 295 300
 Glu Arg Pro Gly Pro Gly Gly Gly Ala Cys Glu Pro Arg Gly Gly Glu
 305 310 315 320
 Pro Ser Ser Gly Pro Val Arg Arg Glu Leu Lys Gln Phe Leu Gly Trp
 325 330 335
 Leu Lys Lys His Ala Tyr Cys Ser Asn Leu Ser Phe Arg Leu Tyr Asp
 340 345 350
 Gln Trp Arg Ala Trp Met Gln Lys Ser His Lys Thr Arg Asn Gln His
 355 360 365
 Arg Thr Arg Gly Ser Cys Pro Arg Ala Asp Gly Ala Arg Arg Glu Val
 370 375 380
 Leu Pro Asp Lys Leu
 385

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 389 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Ala His Thr Ala Val Ile Ser Pro Gln Asp Pro Thr Leu Leu Ile Gly
 1 5 10 15
 Ser Ser Leu Leu Ala Thr Cys Ser Val His Gly Asp Pro Pro Gly Ala
 20 25 30
 Thr Ala Glu Gly Leu Tyr Trp Thr Leu Asn Gly Arg Arg Leu Pro Pro
 35 40 45
 Glu Leu Ser Arg Val Leu Asn Ala Ser Thr Leu Ala Leu Ala Leu Ala
 50 55 60
 Asn Leu Asn Gly Ser Arg Gln Arg Ser Gly Asp Asn Leu Val Cys His
 65 70 75 80
 Ala Arg Asp Gly Ser Ile Leu Ala Gly Ser Cys Leu Tyr Val Gly Leu
 85 90 95
 Pro Pro Glu Lys Pro Val Asn Ile Ser Cys Trp Ser Lys Asn Met Lys
 100 105 110
 Asp Leu Thr Cys Arg Trp Thr Pro Gly Ala His Gly Glu Thr Phe Leu
 115 120 125
 His Thr Asn Tyr Ser Leu Lys Tyr Lys Leu Arg Trp Tyr Gly Gln Asp
 130 135 140

```

Asn Thr Cys Glu Glu Tyr His Thr Val Gly Pro His Ser Cys His Ile
145                150                155                160
Pro Lys Asp Leu Thr Leu Phe Thr Pro Tyr Glu Ile Trp Val Glu Ala
                165                170                175
Thr Asn Arg Leu Gly Ser Ala Arg Ser Asp Val Leu Thr Leu Asp Ile
                180                185                190
Leu Asp Val Val Thr Thr Asp Pro Pro Pro Asp Val His Val Ser Arg
                195                200                205
Val Gly Gly Leu Glu Asp Gln Leu Ser Val Arg Trp Val Ser Pro Pro
                210                215                220
Ala Leu Lys Asp Phe Leu Phe Gln Ala Lys Tyr Gln Ile Arg Tyr Arg
225                230                235                240
Val Glu Asp Ser Val Asp Trp Lys Val Val Asp Asp Val Ser Asn Gln
                245                250                255
Thr Ser Cys Arg Leu Ala Gly Leu Lys Pro Gly Thr Val Tyr Phe Val
                260                265                270
Gln Val Arg Cys Asn Pro Phe Gly Ile Tyr Gly Ser Lys Lys Ala Gly
                275                280                285
Ile Trp Ser Glu Trp Ser His Pro Thr Ala Ala Ser Thr Pro Arg Ser
                290                295                300
Glu Arg Pro Gly Pro Gly Gly Gly Ala Cys Glu Pro Arg Gly Gly Glu
305                310                315                320
Pro Ser Ser Gly Pro Val Arg Arg Glu Leu Lys Gln Phe Leu Gly Trp
                325                330                335
Leu Lys Lys His Ala Tyr Cys Ser Asn Leu Ser Phe Arg Leu Tyr Asp
                340                345                350
Gln Trp Arg Ala Trp Met Gln Lys Ser His Lys Thr Arg Asn Gln His
                355                360                365
Arg Thr Arg Gly Ser Cys Pro Arg Ala Asp Gly Ala Arg Arg Glu Val
                370                375                380
Leu Pro Asp Lys Leu
385

```

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 389 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Ala His Thr Ala Val Ile Ser Pro Gln Asp Pro Thr Leu Leu Ile Gly
 1 5 10 15
 Ser Ser Leu Leu Ala Thr Cys Ser Val His Gly Asp Pro Pro Gly Ala
 20 25 30
 Thr Ala Glu Gly Leu Tyr Trp Thr Leu Asn Gly Arg Arg Leu Pro Pro
 35 40 45
 Glu Leu Ser Arg Val Leu Asn Ala Ser Thr Leu Ala Leu Ala Leu Ala
 50 55 60
 Asn Leu Asn Gly Ser Arg Gln Arg Ser Gly Asp Asn Leu Val Cys His
 65 70 75 80
 Ala Arg Asp Gly Ser Ile Leu Ala Gly Ser Cys Leu Tyr Val Gly Leu
 85 90 95
 Pro Pro Glu Lys Pro Val Asn Ile Ser Cys Trp Ser Lys Asn Met Lys
 100 105 110
 Asp Leu Thr Cys Arg Trp Thr Pro Gly Ala His Gly Glu Thr Phe Leu
 115 120 125
 His Thr Asn Tyr Ser Leu Lys Tyr Lys Leu Arg Trp Tyr Gly Gln Asp
 130 135 140
 Asn Thr Cys Glu Glu Tyr His Thr Val Gly Pro His Ser Cys His Ile
 145 150 155 160
 Pro Lys Asp Leu Ala Leu Phe Thr Pro Tyr Glu Ile Trp Val Glu Ala
 165 170 175
 Thr Asn Arg Leu Gly Ser Ser Arg Ser Asp Val Leu Thr Leu Asp Ile
 180 185 190
 Leu Asp Val Val Thr Thr Asp Pro Pro Pro Asp Val His Val Ser Arg
 195 200 205
 Val Gly Gly Leu Glu Asp Gln Leu Ser Val Arg Trp Val Ser Pro Pro
 210 215 220
 Ala Leu Lys Asp Phe Leu Phe Gln Ala Lys Tyr Gln Ile Arg Tyr Arg
 225 230 235 240
 Val Glu Asp Ser Val Asp Trp Lys Val Val Asp Asp Val Ser Asn Gln
 245 250 255
 Thr Ser Cys Arg Leu Ala Gly Leu Lys Pro Gly Thr Val Tyr Phe Val
 260 265 270
 Gln Val Arg Cys Asn Pro Phe Gly Ile Tyr Gly Ser Lys Lys Ala Gly
 275 280 285
 Ile Trp Ser Glu Trp Ser His Pro Thr Ala Ala Ser Thr Pro Arg Ser
 290 295 300
 Glu Arg Pro Gly Pro Gly Gly Gly Ala Cys Glu Pro Arg Gly Gly Glu
 305 310 315 320
 Pro Ser Ser Gly Pro Val Arg Arg Glu Leu Lys Gln Phe Leu Gly Trp
 325 330 335
 Leu Lys Lys His Ala Tyr Cys Ser Asn Leu Ser Phe Arg Leu Tyr Asp
 340 345 350

Gln Trp Arg Ala Trp Met Gln Lys Ser His Lys Thr Arg Asn Gln His
 355 360 365
 Arg Thr Arg Gly Ser Cys Pro Arg Ala Asp Gly Ala Arg Arg Glu Val
 370 375 380
 Leu Pro Asp Lys Leu
 385

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 389 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Ala His Thr Ala Val Ile Ser Pro Gln Asp Pro Thr Leu Leu Ile Gly
 1 5 10 15
 Ser Ser Leu Leu Ala Thr Cys Ser Val His Gly Asp Pro Pro Gly Ala
 20 25 30
 Thr Ala Glu Gly Leu Tyr Trp Thr Leu Asn Gly Arg Arg Leu Pro Pro
 35 40 45
 Glu Leu Ser Arg Val Leu Asn Ala Ser Thr Leu Ala Leu Ala Leu Ala
 50 55 60
 Asn Leu Asn Gly Ser Arg Gln Arg Ser Gly Asp Asn Leu Val Cys His
 65 70 75 80
 Ala Arg Asp Gly Ser Ile Leu Ala Gly Ser Cys Leu Tyr Val Gly Leu
 85 90 95
 Pro Pro Glu Lys Pro Val Asn Ile Ser Cys Trp Ser Lys Asn Met Lys
 100 105 110
 Asp Leu Thr Cys Arg Trp Thr Pro Gly Ala His Gly Glu Thr Phe Leu
 115 120 125
 His Thr Asn Tyr Ser Leu Lys Tyr Lys Leu Arg Trp Tyr Gly Gln Asp
 130 135 140
 Asn Thr Cys Glu Glu Tyr His Thr Val Gly Pro His Ser Cys His Ile
 145 150 155 160
 Pro Lys Asp Leu Ala Leu Phe Thr Pro Tyr Glu Ile Trp Val Glu Ala
 165 170 175
 Thr Asn Arg Leu Gly Ser Ala Arg Ser Asp Val Leu Thr Leu Asp Val
 180 185 190
 Leu Asp Val Val Thr Thr Asp Pro Pro Pro Asp Val His Val Ser Arg
 195 200 205

Val Gly Gly Leu Glu Asp Gln Leu Ser Val Arg Trp Val Ser Pro Pro
 210 215 220
 Ala Leu Lys Asp Phe Leu Phe Gln Ala Lys Tyr Gln Ile Arg Tyr Arg
 225 230 235 240
 Val Glu Asp Ser Val Asp Trp Lys Val Val Asp Asp Val Ser Asn Gln
 245 250 255
 Thr Ser Cys Arg Leu Ala Gly Leu Lys Pro Gly Thr Val Tyr Phe Val
 260 265 270
 Gln Val Arg Cys Asn Pro Phe Gly Ile Tyr Gly Ser Lys Lys Ala Gly
 275 280 285
 Ile Trp Ser Glu Trp Ser His Pro Thr Ala Ala Ser Thr Pro Arg Ser
 290 295 300
 Glu Arg Pro Gly Pro Gly Gly Gly Ala Cys Glu Pro Arg Gly Gly Glu
 305 310 315 320
 Pro Ser Ser Gly Pro Val Arg Arg Glu Leu Lys Gln Phe Leu Gly Trp
 325 330 335
 Leu Lys Lys His Ala Tyr Cys Ser Asn Leu Ser Phe Arg Leu Tyr Asp
 340 345 350
 Gln Trp Arg Ala Trp Met Gln Lys Ser His Lys Thr Arg Asn Gln His
 355 360 365
 Arg Thr Arg Gly Ser Cys Pro Arg Ala Asp Gly Ala Arg Arg Glu Val
 370 375 380
 Leu Pro Asp Lys Leu
 385

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 389 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Ala His Thr Ala Val Ile Ser Pro Gln Asp Pro Thr Leu Leu Ile Gly
 1 5 10 15
 Ser Ser Leu Leu Ala Thr Cys Ser Val His Gly Asp Pro Pro Gly Ala
 20 25 30
 Thr Ala Glu Gly Leu Tyr Trp Thr Leu Asn Gly Arg Arg Leu Pro Pro
 35 40 45
 Glu Leu Ser Arg Val Leu Asn Ala Ser Thr Leu Ala Leu Ala Leu Ala
 50 55 60

Asn Leu Asn Gly Ser Arg Gln Arg Ser Gly Asp Asn Leu Val Cys His
 65 70 75 80
 Ala Arg Asp Gly Ser Ile Leu Ala Gly Ser Cys Leu Tyr Val Gly Leu
 85 90 95
 Pro Pro Glu Lys Pro Val Asn Ile Ser Cys Trp Ser Lys Asn Met Lys
 100 105 110
 Asp Leu Thr Cys Arg Trp Thr Pro Gly Ala His Gly Glu Thr Phe Leu
 115 120 125
 His Thr Asn Tyr Ser Leu Lys Tyr Lys Leu Arg Trp Tyr Gly Gln Asp
 130 135 140
 Asn Thr Cys Glu Glu Tyr His Thr Val Gly Pro His Ser Cys His Ile
 145 150 155 160
 Pro Lys Asp Leu Ala Leu Phe Thr Pro Tyr Glu Ile Trp Val Glu Ala
 165 170 175
 Thr Asn Arg Leu Gly Ser Ala Arg Ser Asp Val Leu Thr Leu Asp Ile
 180 185 190
 Leu Asp Ile Val Thr Thr Asp Pro Pro Pro Asp Val His Val Ser Arg
 195 200 205
 Val Gly Gly Leu Glu Asp Gln Leu Ser Val Arg Trp Val Ser Pro Pro
 210 215 220
 Ala Leu Lys Asp Phe Leu Phe Gln Ala Lys Tyr Gln Ile Arg Tyr Arg
 225 230 235 240
 Val Glu Asp Ser Val Asp Trp Lys Val Val Asp Asp Val Ser Asn Gln
 245 250 255
 Thr Ser Cys Arg Leu Ala Gly Leu Lys Pro Gly Thr Val Tyr Phe Val
 260 265 270
 Gln Val Arg Cys Asn Pro Phe Gly Ile Tyr Gly Ser Lys Lys Ala Gly
 275 280 285
 Ile Trp Ser Glu Trp Ser His Pro Thr Ala Ala Ser Thr Pro Arg Ser
 290 295 300
 Glu Arg Pro Gly Pro Gly Gly Gly Ala Cys Glu Pro Arg Gly Gly Glu
 305 310 315 320
 Pro Ser Ser Gly Pro Val Arg Arg Glu Leu Lys Gln Phe Leu Gly Trp
 325 330 335
 Leu Lys Lys His Ala Tyr Cys Ser Asn Leu Ser Phe Arg Leu Tyr Asp
 340 345 350
 Gln Trp Arg Ala Trp Met Gln Lys Ser His Lys Thr Arg Asn Gln His
 355 360 365
 Arg Thr Arg Gly Ser Cys Pro Arg Ala Asp Gly Ala Arg Arg Glu Val
 370 375 380
 Leu Pro Asp Lys Leu
 385

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 389 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

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Ala His Thr Ala Val Ile Ser Pro Gln Asp Pro Thr Leu Leu Ile Gly
 1           5           10           15
Ser Ser Leu Leu Ala Thr Cys Ser Val His Gly Asp Pro Pro Gly Ala
          20           25           30
Thr Ala Glu Gly Leu Tyr Trp Thr Leu Asn Gly Arg Arg Leu Pro Pro
          35           40           45
Glu Leu Ser Arg Val Leu Asn Ala Ser Thr Leu Ala Leu Ala Leu Ala
          50           55           60
Asn Leu Asn Gly Ser Arg Gln Arg Ser Gly Asp Asn Leu Val Cys His
65           70           75           80
Ala Arg Asp Gly Ser Ile Leu Ala Gly Ser Cys Leu Tyr Val Gly Leu
          85           90           95
Pro Pro Glu Lys Pro Val Asn Ile Ser Cys Trp Ser Lys Asn Met Lys
          100          105          110
Asp Leu Thr Cys Arg Trp Thr Pro Gly Ala His Gly Glu Thr Phe Leu
          115          120          125
His Thr Asn Tyr Ser Leu Lys Tyr Lys Leu Arg Trp Tyr Gly Gln Asp
          130          135          140
Asn Thr Cys Glu Glu Tyr His Thr Val Gly Pro His Ser Cys His Ile
145          150          155          160
Pro Lys Asp Leu Ala Leu Phe Thr Pro Tyr Glu Ile Trp Val Glu Ala
          165          170          175
Thr Asn Arg Leu Gly Ser Ala Arg Ser Asp Val Leu Thr Leu Asp Ile
          180          185          190
Leu Asp Val Val Thr Thr Asp Pro Pro Pro Asp Val His Val Ser Arg
          195          200          205
Val Gly Gly Leu Glu Asp Gln Leu Ser Val Arg Trp Val Ser Pro Pro
          210          215          220
Ala Leu Lys Asp Phe Leu Phe Gln Ala Lys Tyr Gln Ile Arg Tyr Arg
225          230          235          240
Val Glu Asp Ser Val Asp Trp Lys Val Val Asp Asp Val Ser Asn Gln
          245          250          255
Thr Ser Cys Arg Ile Ala Gly Leu Lys Pro Gly Thr Val Tyr Phe Val
          260          265          270

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Gln Val Arg Cys Asn Pro Phe Gly Ile Tyr Gly Ser Lys Lys Ala Gly
 275 280 285
 Ile Trp Ser Glu Trp Ser His Pro Thr Ala Ala Ser Thr Pro Arg Ser
 290 295 300
 Glu Arg Pro Gly Pro Gly Gly Gly Ala Cys Glu Pro Arg Gly Gly Glu
 305 310 315 320
 Pro Ser Ser Gly Pro Val Arg Arg Glu Leu Lys Gln Phe Leu Gly Trp
 325 330 335
 Leu Lys Lys His Ala Tyr Cys Ser Asn Leu Ser Phe Arg Leu Tyr Asp
 340 345 350
 Gln Trp Arg Ala Trp Met Gln Lys Ser His Lys Thr Arg Asn Gln His
 355 360 365
 Arg Thr Arg Gly Ser Cys Pro Arg Ala Asp Gly Ala Arg Arg Glu Val
 370 375 380
 Leu Pro Asp Lys Leu
 385

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 389 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Ala His Thr Ala Val Ile Ser Pro Gln Asp Pro Thr Leu Leu Ile Gly
 1 5 10 15
 Ser Ser Leu Leu Ala Thr Cys Ser Val His Gly Asp Pro Pro Gly Ala
 20 25 30
 Thr Ala Glu Gly Leu Tyr Trp Thr Leu Asn Gly Arg Arg Leu Pro Pro
 35 40 45
 Glu Leu Ser Arg Val Leu Asn Ala Ser Thr Leu Ala Leu Ala Leu Ala
 50 55 60
 Asn Leu Asn Gly Ser Arg Gln Arg Ser Gly Asp Asn Leu Val Cys His
 65 70 75 80
 Ala Arg Asp Gly Ser Ile Leu Ala Gly Ser Cys Leu Tyr Val Gly Leu
 85 90 95
 Pro Pro Glu Lys Pro Val Asn Ile Ser Cys Trp Ser Lys Asn Met Lys
 100 105 110
 Asp Leu Thr Cys Arg Trp Thr Pro Gly Ala His Gly Glu Thr Phe Leu
 115 120 125

100

His Thr Asn Tyr Ser Leu Lys Tyr Lys Leu Arg Trp Tyr Gly Gln Asp
 130 135 140
 Asn Thr Cys Glu Glu Tyr His Thr Val Gly Pro His Ser Cys His Ile
 145 150 155 160
 Pro Lys Asp Leu Ala Leu Phe Thr Pro Tyr Glu Ile Trp Val Glu Ala
 165 170 175
 Thr Asn Arg Leu Gly Ser Ala Arg Ser Asp Val Leu Thr Leu Asp Ile
 180 185 190
 Leu Asp Val Val Thr Thr Asp Pro Pro Pro Asp Val His Val Ser Arg
 195 200 205
 Val Gly Gly Leu Glu Asp Gln Leu Ser Val Arg Trp Val Ser Pro Pro
 210 215 220
 Ala Leu Lys Asp Phe Leu Phe Gln Ala Lys Tyr Gln Ile Arg Tyr Arg
 225 230 235 240
 Val Glu Asp Ser Val Asp Trp Lys Val Val Asp Asp Val Ser Asn Gln
 245 250 255
 Thr Ser Cys Arg Leu Ile Gly Leu Lys Pro Gly Thr Val Tyr Phe Val
 260 265 270
 Gln Val Arg Cys Asn Pro Phe Gly Ile Tyr Gly Ser Lys Lys Ala Gly
 275 280 285
 Ile Trp Ser Glu Trp Ser His Pro Thr Ala Ala Ser Thr Pro Arg Ser
 290 295 300
 Glu Arg Pro Gly Pro Gly Gly Gly Ala Cys Glu Pro Arg Gly Gly Glu
 305 310 315 320
 Pro Ser Ser Gly Pro Val Arg Arg Glu Leu Lys Gln Phe Leu Gly Trp
 325 330 335
 Leu Lys Lys His Ala Tyr Cys Ser Asn Leu Ser Phe Arg Leu Tyr Asp
 340 345 350
 Gln Trp Arg Ala Trp Met Gln Lys Ser His Lys Thr Arg Asn Gln His
 355 360 365
 Arg Thr Arg Gly Ser Cys Pro Arg Ala Asp Gly Ala Arg Arg Glu Val
 370 375 380
 Leu Pro Asp Lys Leu
 385

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 48 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Asn	Ser	Ala	Arg	Gly	Ala	Cys	Val	Pro	Arg	Arg	Ala	Pro	Pro	Pro	Pro
1				5				10					15		
Ser	Arg	Ser	Pro	Pro	Arg	Ala	Pro	Gly	Ser	Ala	Gly	Pro	Met	Pro	Ala
			20					25					30		
Gly	Pro	Met	Pro	Ala	Gly	Arg	Arg	Gly	Pro	Ala	Ala	Gln	Ser	Ala	Arg
		35					40						45		

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Asn	Ser	Ala	Arg	Gly	Ala	Cys	Val	Pro	Arg	Arg	Ala	Pro	Pro	Pro	Pro
1				5				10					15		
Ser	Arg	Ser	Pro	Pro	Arg	Ala	Pro	Gly	Ser						
			20					25							

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Ala	Gly	Pro	Met	Pro	Ala	Gly	Pro	Met	Pro	Ala	Gly	Arg	Arg	Gly	Pro
1				5				10					15		
Ala	Ala	Gln	Ser	Ala	Arg										
			20												

(2) INFORMATION FOR SEQ ID NO:35:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Ser	Pro	Pro	Ala	Leu	Lys	Asp	Phe	Leu	Phe	Gln	Ala	Lys	Tyr	Gln	Ile
1				5				10					15		
Arg	Tyr	Arg	Val	Glu	Asp	Ser	Val	Asp	Trp	Lys	Val	Val	Asp	Asp	Val
			20					25					30		

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Ala	Ala	Ser	Thr	Pro	Arg	Ser	Glu	Arg	Pro	Gly	Pro	Gly	Gly	Gly	Ala
1				5				10					15		
Cys	Glu	Pro	Arg	Gly	Gly	Glu	Pro	Ser	Ser	Gly	Pro	Val	Arg	Arg	
			20					25					30		

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Trp	Met	Gln	Lys	Ser	His	Lys	Thr	Arg	Asn	Gln	Asp	Glu	Gly	Ile	Leu
1				5				10					15		
Pro	Ser	Gly	Arg	Arg	Gly	Thr	Ala	Arg	Gly	Pro	Ala	Arg			
			20					25							

CLAIMS

We claim:

1. An isolated polynucleotide which encodes a mammalian polypeptide, said polypeptide being comprised of an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, and SEQ ID NOS: 17 - 37 or a polypeptide which is 90%, 95% or 99% identical to said amino acid sequences.

2. The isolated polynucleotide of claim 1 wherein said polynucleotide is a DNA sequence.

3. The isolated polynucleotide of claim 1 wherein said polynucleotide is an RNA sequence.

4. An expression vector comprising the following operably linked elements:

a transcription promoter;

a DNA segment encoding a mammalian polypeptide, said polypeptide being comprised of an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NOS: 17 - 37; and

a transcription terminator.

5. An isolated polypeptide said polypeptide being comprised of an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, and SEQ ID NOS: 17 - 31 or a polypeptide which is 90%, 95% or 99% identical to said amino acid sequences.

6. A peptide or polypeptide which has the amino acid sequence of an epitope-bearing portion of a Zcytor5 polypeptide.

7. A polypeptide of claim 6 wherein the polypeptide has amino acid sequence of at least 15 amino acid residues.

8. The polypeptide of claim 7 wherein said polypeptide is selected from the group of polypeptide consisting of the amino acid sequences of SEQ ID NOs: 32-37.

9. An antibody which specifically binds to an epitope-binding sequence of a Zcytor5 polypeptide.

10. An antibody of claim 9 wherein said antibody binds to a polypeptide comprised of an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, and SEQ ID NOs: 17 - 37.

11. An anti-idiotypic antibody of an antibody of claims 9 or 10.

12. A method for producing an antibody which binds to a Zcytor5 polypeptide comprising inoculating an animal with an epitope-bearing amino acid sequence of Zcytor5 polypeptide under conditions wherein said animal produces antibodies which bind to the Zcytor5 polypeptide; and
isolating said antibodies.

INTERNATIONAL SEARCH REPORT

Inventor's Name: International Application No

PCT/US 98/08865

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/715 C07K16/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE EMBL</p> <p>Accession Nbr W66776, 15 June 1996</p> <p>MARRA M ET AL: "me17b11.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 387741 5' similar to PIR:B38252 B38252 granulocyte colony-stimulating factor receptor precursor ;."</p> <p>XP002075171</p> <p>97.9% identity in 96AA overlap between translated sequence and SEQ ID 2</p> <p>99.0% identity in 96AA overlap between translated sequence and SEQ ID 4</p> <p>100% identity in 96AA overlap between translated sequence and SEQ ID 6</p> <p style="text-align: center;">---</p> <p style="text-align: center;">-/--</p>	1-3

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

25 August 1998

Date of mailing of the international search report

07/09/1998

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Authorized officer

Lejeune, R

INTERNATIONAL SEARCH REPORT

Int. ational Application No

PCT/US 98/08865

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE EMBL Accession Nbr AA049280, 29 November 1996 MARRA M ET AL: "mj45d02.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 479043 5' similar to SW:IL6B_MOUSE Q00560 INTERLEUKIN-6 RECEPTOR BETA CHAIN PRECURSOR ;."</p> <p>XP002075172 98.1% identity in 154AA overlap between translated sequence and SEQ ID 2 98.7% identity in 154AA overlap between translated sequence and SEQ ID 4 100% identity in 154AA overlap between translated sequence and SEQ ID 6</p> <p>----</p>	1-3
P,X	<p>WO 98 11225 A (NICOLA NICOS ANTONY ;FABRI LOUIS (AU); FARLEY ALISON (AU); NASH AN) 19 March 1998 see abstract 94.6% identity in 424 AA overlap between SEQ ID 15 (pages 87-90) and SEQ ID 2 92.4% identity in 421 AA overlap between SEQ ID 15 (pages 87-90) and SEQ ID 4 98.8% identity in 425 AA overlap between SEQ ID 15 (pages 87-90) and SEQ ID 6</p> <p>----</p>	1-8
E	<p>WO 98 31811 A (DONALDSON DEBRA D ;GENETICS INST (US); COLLINS MARY (US); NEBEN TA) 23 July 1998 see abstract 99.8% identity in 408 AA overlap between SEQ ID 7 (pages 29-30) and SEQ ID 2 99.7% identity in 390 AA overlap between SEQ ID 7 (pages 29-30) and SEQ ID 4 98.6% identity in 425 AA overlap between SEQ ID 5 (pages 26-27) and SEQ ID 6</p> <p>----</p>	1-8
T	<p>ELSON G C ET AL: "Cytokine-like factor-1, a novel soluble protein, shares homology with members of the cytokine type I receptor family." JOURNAL OF IMMUNOLOGY, vol. 161, no. 3, 1 August 1998, pages 1371-1379, XP002075165 see abstract see figure 1</p> <p>-----</p>	1-8

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/08865

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9811225 A	19-03-1998	AU 4308097 A	02-04-1998
WO 9831811 A	23-07-1998	NONE	